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Abstract Book

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Society for Endocrinology Dale Medal Lecture

Ronald M Evans, The Salk Institute for Biological Studies, LaJolla California, USA

Dr. Ronald M Evans is known for his discoveries and characterization of nuclear hormone receptors, the establishment of the nuclear receptor super family and the elucidation of their universal mechanism of action, this revealed how receptor activation by lipophilic hormones and drugs are transformed into physiology and the treatment of disease.

Dr R M Evans obtained his BA and PhD from the University of California, Los Angeles, School of Medicine in 1970 and 1974 respectively, was a postdoctoral fellow with James Darnell at the Rockefeller University in New York and in 1977 joined the faculty of The Salk Institute for Biological Studies where he is now an Investigator of the Howard Hughes Medical Institute and Professor in the Gene Expression Laboratory. He holds the March of Dimes Chair in Molecular and Developmental Biology and is Adjunct Professorships at the University of California, San Diego in the Departments of Biology, Biomedical Sciences, and Neuroscience.

At Salk, Dr Evans isolated the GH gene to study its transcriptional regulation by steroid and thyroid hormones. In 1985 his group cloned and characterized the first nuclear hormone receptor, the human glucocorticoid receptor. His subsequent isolation of the thyroid, mineralocorticoid and retinoic acid (vitamin A) established the existence of the nuclear receptor superfamily. This work led to the principles of DNA recognition, receptor heterodimer formation, and the discovery of the DNA coding mechanism for hormone response elements. He isolated the first orphan receptors (ERR1and 2) as well as the unexpectedly important retinoid X receptor (RXR). He pioneered biochemical and molecular techniques (termed reverse endocrinology) that led to the identification of the RXR ligand 9-*cis* RA. RXR proved to be a Rosetta stone for puzzling out the identity of a series of unknown receptors, which have profound implications for normal physiology, disease pathogenesis and drug discovery. He also isolated and characterized the xenobiotic sensor SXR.

More recently, Dr Evans has focused on PPARg and d as major regulators of whole body lipid metabolism. As part of this work he created genetically thin mice and the first animal (termed the 'Marathon Mouse') genetically engineered for increased running endurance. This led to the recent discovery that transcription of a nuclear gene network by a PPARδ synthetic agonist and the AMP kinase (AMPK) activator 'AICAR' can enhance running endurance in absence of mechanical exercise. More recently, he has extended this concept by demonstrating that AICAR can act like 'pharmacologic light' to entrain the rhythm of the hepatic circadian clock.

Selected awards since 2000 include: 1st Bristol – Myers Squibb Award for Metabolic Research (2000), City of Medicine Award, Duke (2002), the March of Dimes Prize in Developmental Biology (2003), General Motors Cancer Research Foundation Alfred P Sloan Medal (2003), Keio Medical Science Prize (2003), Albert Lasker Basic Medical Research Award (2004), Glen T Seaborg Medal, UCLA (2005), 'Grande Medaille d'Or' of the French Academy of Sciences (2005), Gairdner Award, Canada (2006), Harvey Prize of the Technion Institute, Israel (2006), the Albany Prize in Medicine (2007) and the Endocrine Regulation Prize, IPSEN Foundation (2008), Ernst Knobil Award, U Texas Hlth Sci Cntr (2009), Wolf Prize, Wolf Foundation Israel (2012), Dale Medal, British Society for Endocrinology, UK (2013). He is a member of the National Academy of Sciences, the Institute of Medicine, the American Academy of Arts and Sciences, the American Philosophical Society and was named the1994 California Scientist of the Year.

Society for Endocrinology Hoffenberg International Medal Lecture

Fernand Labrie (Professor), Department of Anatomy and Physiology, Laval university, Quebec City Quebec, Canada

After obtaining his MD and PhD (Endocrinology) degrees with Honours at Laval University, Quebec City, Canada, F Labrie pursued his postdoctoral training at the University of Cambridge, UK, first in the Laboratory of Professor Asher Korner and then, in the Laboratory of Molecular Biology of Professor Frederick Sanger, twice Nobel laureate in medicine. Dr Labrie then isolated the first mammalian messenger RNA before returning to Laval University in 1969 where he founded the Laboratory of Molecular Endocrinology, one of the largest research groups in endocrinology worldwide with a total personnel of up to 350 members including 32 senior scientists. Between 1982 and 2008, he has been scientific director of the CHUL Research Center (1200 employees), one of the largest medical research Institutes in Canada. From 1990 to 2002, Dr Labrie has been head of the Department of Anatomy and Physiology of the Faculty of Medicine at Laval University while between 1992 and 1995, he has been president of the Fonds de la Recherche en Santé du Québec.

The most important contribution of Dr Labrie to clinical medicine has been the discovery and development of medical castration with GnRH agonists as well as combined androgen blockade, the first treatment shown to prolong life in prostate cancer and at the basis of the recent developments using blockade of androgens made locally in the prostate in castration – resistant prostate cancer. GnRH agonists and combined androgen blockade have become the standard hormonal therapy of prostate cancer worldwide. He also discovered that a large proportion of androgens and estrogens in women (100% after menopause) and men are made in peripheral tissues from dehydroepiandrosterone by the mechanism of intracrinology. Dr Labrie and his group then discovered the most potent antiestrogen, namely Acolbifene, and performed all related toxicology, phases I and II clinical studies.

Dr Labrie's discoveries are described in more than 1250 scientific publications and have been cited more than 40 000 times. Dr Labrie is the most cited Canadian scientist among all disciplines in the international literature. He recently won the King Faisal International Prize in medicine. He received numerous other awards, including the Friesen Award of the Canadian Society of Clinical Investigation and is Doctor Honoris Causa at the Universities of Caen and Athens.

Society for Endocrinology European Medal Lecture

Anna Spada, Full Professor of Endocrinology, School of Medicine, University of Milan, Milan, Italy

Anna Spada is currently Full Professor at the School of Medice, University of Milan. Her main research interests are on signal transduction in pituitary cells, pathogenesis of pituitary tumors, genotype/phenotype relationships in acromegalic patients with gsp mutations, tissue specific GNAS1 gene imprinting, molecular mechanisms of resistance to hormone action, activating and inactivating mutations of GNAS1 in endocrine disorders, polymorphic variants of somatostatin receptor genes in acromegalic patients, polymorphic variants of D2 receptor gene and resistance to cabergoline, pharmacogenomics.

Professor A Spada has been on the Editorial Board of numerous, prestigious peer-revied journal including *Endocrinology, Journal of Endocrinological Investigation, Endocrine-Related Cancer* and is past Editor-in-Chief of the *Journal of Molecular Endocrinology*.

She has chared 98 national and 42 International Meetings and has lectured world-wide. Professor A Spada has authored over 133 articles in peer-reviewed journals.

Society for Endocrinology Medal Lecture

Marta Korbonits, Department of Endocrinology, St Barts and the London School of Medicine, London, UK

Professor Korbonits is a clinical academic endocrinologist with special interest in pituitary tumorigenesis and as well as metabolic effects of hormones. She graduated in medicine at Semmelweis Medical School in Budapest and works in the Department of Endocrinology at Barts and the London School of Medicine at St. Bartholomew's Hospital in London since 1991, where currently she is Co-Centre Head. She received and MD and a PhD from the University of London and was a recipient of an MRC Clinician Scientist Fellowship to study ghrelin physiology and genetics. Her current interest include hormonal regulation of the metabolic enzyme AMP-activated protein kinase, the physiology and pathophysiology of ghrelin and endocannabinoids and pituitary tumours including familial cases. She has a large collection of familial isolated pituitary adenoma families and works on both the clinical characterisation as well as molecular aspects of this disease.

She has published over 150 papers, numerous book chapters, and edited books in the filed of Endocrinology and serves on the editorial board of several prestigious endocrine journals. She was heading the Program Organising Committee of the Society for Endocrinology for 3 years, and currently serves on the Executive Committee of the Pituitary Society and the European Society of Clinical Investigation and is an elected member of the Association of Physicians of Great Britain and Ireland.

She shares her time between clinical patient care, clinical research and laboratory based research as well as teaching at undergraduate and postgraduate level.

Clinical Endocrinology Trust Lecture

Stafford Lightman, Professor of Medicine, University of Bristol, Bristol, UK

Stafford Lightman is Professor of Medicine at the University of Bristol and is Director of the Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology. He started his scientific career working on catecholamines and opioid peptides with Leslie Iversen at the University of Cambridge and provided some of the first data linking opioid peptides with the regulation of neurohypophysial function. At this time he also performed some of the first studies demonstrating the importance of brain stem catecholamine pathways in the regulation of hypothalamic activity. On moving to what is now Imperial College in London, he started to develop his studies on the role of the brain in the regulation of stress response. He demonstrated the shift from CRH to arginine vasopressin in the control of the hypothalamic-pituitary-adrenal axis during chronic stress, demonstrated and characterised the development of stress hyporesponsiveness during lactation in both rats and man and developed models of immunological activation of the stress response. More recently he has developed the concept of emergent pulsatility of hormone secretion as a result of inherent delays in the feedforward or feedback relationships regulating endocrine activity. This has also led to a new emphasis on the importance of digital signalling at the level of glucocorticoid receptors and GR chromatin interactions.

S Lightman was the founder Editor-in-Chief of the Journal of Neuroendocrinology, a founder Fellow of the Faculty of Medical Sciences, the founder Chairman of the Pituitary Foundation and a Council Member of the Physiological Society. He sits on several Research Councils, Wellcome Trust and European Research Committees and has Chaired the European Union Committee Review of Tertiary Education in East Africa. Professor Lightman also has a major interest in inter-relationships between art and neuroscience.

Clinical Endocrinology Trust Visiting Professor Lecture

Lynnette Nieman, National Institutes of Health, Bethesda, Maryland, USA

Dr Lynnette Nieman is a Senior Investigator and Chief of the Endocrinology Consultation Service at the National Institutes of Health (NIH) Clinical Research Center. She has been at the NIH since her fellowship. From 1991 to 2001 she served as the Clinical Director of intramural NICHD, overseeing clinical care of the institute's patients and ensuring compliance with human subjects research regulations.

Dr Nieman is an active clinician, having seen more than 1100 patients with Cushing's syndrome and is the Principal Investigator for six active protocols. She has authored more than 250 publications and sponsored three investigational new drug applications to the FDA, one of which was licensed in the US and Europe. She provided Congressional testimony on one of these agents. She is a co-editor of the Adrenal Section of UpToDate and an associate editor for the Journal of Clinical Endocrinology and Metabolism.

She is a member of the subcommittee that creates the US Endocrinology and Metabolism certification examination. Dr Nieman has received the NIH Director's Award, Clinical Teacher of the Year award and the Endocrine Society's Distinguished Physician award. She is a past Vice President for Clinical Science of the Endocrine Society and Chaired its 2012 annual meeting.

Plenary Lectures

Society of Endocrinology Dale Medal Lecture
PL1

Nuclear receptors and AMPK: can exercise mimetics cure diabetes

Ronald Evans
Salk Institute for Biological Studies, La Jolla, CA, USA.

Nuclear hormone receptors (NHRs) are a large family of ligand-activated transcription factors that regulate programs of cellular growth, differentiation and homeostasis. The structurally conserved ligand binding domains (LBDs) of NHRs bind to hydrophobic small molecules including steroid hormones, fat soluble vitamins and bile acids, thereby interpreting small molecule cues to affect transcriptional readouts.

The temporal correspondence between metabolic and circadian rhythms suggests the inherent coupling of these two key physiologic processes. Sleep, inactivity and fasting are opposed by wakefulness, motivated behavior and the fed state. Thus we are interested whether there may be common mechanism for 'entraining' both the clock and key metabolic pathways. We provide evidence that the energy sensor AMPK, via actions as an atypical transcriptional regulator, may function as one such dual entrainment trigger.

In regards to the clock, we provide genetic, mechanistic and pharmacologic evidence that AMPK-dependent phosphorylation enables cryptochrome (e.g. Cry1) to act as energy sensor for metabolic entrainment of the circadian clock. We show that Cry1 acts as a glucocorticoid receptor (GR) repressor and thus controls cyclic glucose production from the liver. In addition, we find that in muscle, usually active PPARd agonists (such as GW1516) are able to promote increased running endurance, suggesting the potential of drugs that can promote the benefits of exercise even in sedentary mice. Finally, we show that the AMPK agonist AICAR is a prototypic "exercise mimetic,' enhancing endurance by stimulating mitochondrial function in muscle. Pharmacologic exercise from AMPK has important therapeutic implications in metabolic disease, atherosclerosis and frailty, as well as an already realized potential for athletic abuse.

Declaration of funding
This work was supported by the National Institute of Health (grant DK057978-32), the Glenn Foundation, the Ellison Medical Foundation and the Helmsley Charitable Trust. Dr Evans is an investigator of the Howard Hughes Medical Institute and March of Dimes Chair in Molecular and Developmental Biology at the Salk Institute.

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Society of Endocrinology Hoffenberg International Medal Lecture
PL2

Multiple applications of intracrinology in clinical medicine

Fernand Labrie[1,2]
[1]EndoCeutics Inc., Quebec City, Quebec, Canada; [2]Emeritus Professor, Laval University, Quebec City, Quebec, Canada.

Man is unique, with some other primates, in having adrenals that secrete large amounts of dehydroepiandrosterone (DHEA). The problem with DHEA, however, especially for women, is that its secretion from the adrenals starts decreasing at the age of 30 years and has already declined, on average, by 60% at menopause. Since there is no other source of sex steroids after menopause than those made locally in peripheral tissues by the mechanisms of intracrinology, it is logical to believe that low DHEA is responsible for the series of medical problems classically associated with the hormone deficiency of postmenopause. As strong support for the mechanism of intracrinology, recent randomized and placebo-controlled studies have shown that all the signs and symptoms of vulvovaginal atrophy can be rapidly improved or corrected by local administration of DHEA without systemic exposure to estrogens. In men, the combination of a pure antiandrogen with a GnRH agonist was the first treatment shown to prolong life in prostate cancer and can cure the disease in most cases if treatment is started at the localized stage. As a follow-up to our initial observations on the dual source of androgens and role of intracrinology in men, positive clinical data have been obtained with the new antiandrogen MDV3100 as well as with abiraterone, an inhibitor of 17α-hydroxylase (CYP17A1) in patients with prostate cancer progressing after castration, a benefit necessarily due to blockade of extratesticular androgens made in the prostate by intracrinology. On the other hand, the benefits of aromatase inhibitors and antiestrogens in breast cancer in postmenopausal women are necessarily secondary to the inhibition of the formation and action, respectively, of the estrogens made locally in the breast by the process of intracrinology.

In men, the combination of a pure antiandrogen with a GnRH agonist was the first treatment shown to prolong life in patients with prostate cancer and can cure the disease in most cases if treatment is started at the localized stage. As a follow-up to our initial observations on the dual source of androgens in men, positive clinical data have recently been obtained in studies with the new antiandrogen MDV3100 as well as with abiraterone, an inhibitor of 17α-hydroxylase (CYP17A1) in patients with prostate cancer progressing after castration, a benefit necessarily due to blockade of extratesticular androgens made in the prostate by intracrinology. On the other hand, the benefits of aromatase inhibitors and antiestrogens in breast cancer in postmenopausal women are necessarily secondary to the inhibition of the formation and action, respectively, of the estrogens made locally in the breast by the process of intracrinology.

Declaration of interest
President of EndoCeutics, Inc. Developping new DHEA medical indications.
Declaration of funding
This work was supported by EndoCeutics Inc.

DOI: 10.1530/endoabs.31.PL2

Society of Endocrinology European Medal Lecture
PL3

cAMP in the pituitary: an old messenger for multiple signals

Anna Spada
University of Milan, Milan, Italy.

cAMP is implicated in the regulation of a variety of cell functions that are related to activation of multiple intracellular pathways. In addition to the control of differentiated functions, such as hormone secretion, cAMP inhibits or stimulates cell proliferation depending on the cell type. In particular, consistent with the frequent expression of somatic mutations constitutively activating Gs alpha subunit (gsp oncogene) in GH-secreting adenomas, the activation of cAMP dependent pathway generates proliferative signals in somatotrophs. Conversely, this stimulatory effect is not present, or even reverted in an inhibitory one, in pituitary cells of the lactotroph and gonadotroph lineages, such as human prolactinomas and non-functioning adenomas, as well as the corresponding cell lines (MMQ and HP75). The discrepant responsiveness to cAMP increase is restricted to cell proliferation and cell cycle protein induction, since the stimulatory effects on hormone secretion are maintained in all cell types. Although cAMP effects were initially attributed to protein phosphorylation through the activation of protein kinase A (PKA), other factors, such as the two cAMP-activated guanine nucleotide exchange factors (Epac one and two), have recently been identified as allosteric modulators of cAMP action. While the role of Epac induced activation of Rap1 has been investigated in other endocrine cell systems, such as the thyroid and the adrenal, the impact of this pathway on pituitary cells is still undefined. Recent data in the lab indicate that the stimulatory effect of cAMP on somatotrophs proliferation and the inhibitory effect on lactotrophs and gonadotrophs growth are mimicked by the PKA- and Epac-selective cAMP analogs. Moreover, these agents act synergistically in regulating cell proliferation and hormone secretion. These data rule out the involvement of Epac-generated signals on the divergent effects of cAMP in pituitary cells of different types, suggesting possible different expression and/or function of the cAMP and Ras-Raf-ERK cross-signalling components.

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Society of Endocrinology Tranatlantic Medal Lecture
PL4

Abstract unavailable.

DOI: 10.1530/endoabs.31.PL4

British Thyroid Association Pitt-Rivers lecture

PL5

BTA_Pitt Rivers Lecture

Antonio C Bianco
Professor of Medicine, University of Miami, Miami, Florida, USA.

A C Bianco is a professor of medicine and Chief of the Division of Endocrinology, Diabetes and Metabolism at the University of Miami Miller School of Medicine. Dr A C Bianco obtained his MD, PhD and clinical training in internal medicine and endocrinology in Sao Paulo, Brazil. His work has established the importance of the local control of thyroid hormone activation/inactivation via deiodination, as well as fundamental cellular and molecular properties of the deiodinases (D1, D2 and D3). He has also helped to elucidate the three-dimensional structure of the deiodinase-ubiquitination complex, demonstrating that ubiquidation-deubiquidation controls local T_3 production by affecting D2 dimerization. This constitutes a posttranslational on/off switch controlling thyroid hormone action in the settings of development, health and disease.

Partly supported by the ***Clinical Endocrinology Trust***
DOI: 10.1530/endoabs.31.PL5

Society of Endocrinology Medal Lecture

PL6

Genes and giants

Márta Korbonits
Barts and the London School of Medicine, London, UK.

The number of diseases associated with genetic abnormalities has grown exponentially in the last decade. Pituitary tumours are no exception, as now at least nine genes are known to predispose to pituitary tumour development: *MEN1, PRKAR1A, AIP, CDKN1B, SDH (A, B, C and D)* and *DICER1*. On the other hand, only a small minority of the pituitary-related gene carriers develop pituitary disease, suggesting that other interfering genes or factors are also important. Based on our recent assessment in a tertiary referral centre, up to 7% of patients with pituitary adenomas have a family history.

About 20% of familial isolated pituitary adenoma patients have a germline mutation in the *AIP* gene. These patients have a characteristic phenotype with young-onset, usually somatotroph adenoma which is difficult to control with surgery or medical therapy. We have identified a novel pathway involving somatostatin analogues and AIP: somatostatin analogues increase AIP expression and this, in turn, upregulates the transcription factor ZAC1, known to harbour tumour suppressor activity. This mechanism may explain the poor effect of somatostatin analogues in *AIP* mutation-positive patients. *AIP* is a well-conserved gene and its importance is supported by our recent data involving *CG1847*, the fruitfly orthologue of *AIP*. Complete *CG1847* knockdown leads to lethality but organ-specific knockdown can reveal novel AIP interacting partners.

A seemingly far-fetched link between historical patients suffering from gigantism and current families with childhood-onset acromegaly led to the identification of an *AIP* mutation which now ties together 17 kindreds with over 80 carriers. Prospective identification of pituitary disease is emerging as a real possibility, which could potentially eradicate the development of gigantism in these families. Thus, the analysis of genetic syndromes associated with pituitary tumours may shed important light on tumour pathogenesis, and can have a significant impact on patient care.

Declaration of funding
This work was supported by MRC, Wellcome Trust, Barts and the London Charity and Unrestricted educational grant from Pfizer.
DOI: 10.1530/endoabs.31.PL6

Clinical Endocrinology Trust Lecture

PL7

The dynamics of hypothalamo-pituitary-adrenal activity

Stafford Lightman
University of Bristol, Bristol, UK.

The circadian variation of hypothalamo-pituitary-adrenal (HPA) activity is well recognised, with levels of glucocorticoid rising in anticipation of the activity of the coming day (in humans) or night (in rodents). Less well recognised however, is that in common with many other hormones, both ACTH and corticosteroids are released in a pulsatile pattern – with the largest pulses occurring in the morning in man – explaining the large range of 'normal' morning cortisol levels. Although this pulsatility was always assumed to be a result of an undefined pacemaker in the hypothalamus, there is no good experimental data to support this. We have mathematically modelled this system and find that the feedforward:feedback relationship between pituitary corticotrophs and adrenal fasciculata cells obviates the need for an external pacemaker and that the system should oscillate irrespective of the pattern of CRH input. We have now tested this *in vivo* in the rat and have shown that a constant infusion of CRH is sufficient to activate normal ultradian rhythmicity of both ACTH and corticosterone, occurring with the same frequency as that found under normal physiological conditions.

Since pulsatile release of glucocorticoids emerges as fundamental property of the pituitary adrenal system, it would be expected that tissue responses to glucocorticoids have also adapted to make use of this oscillating signal. We have shown that glucocorticoid receptor signalling is indeed able to respond to pulses of corticosterone with pulses of gene transcription (gene pulsing) and that this provides scope for a system that is very responsive to rapid changes in hormone levels – a very important factor for a stress-responding homeostatic system like the HPA axis.

Declaration of funding
The Wellcome Trust, MRC and BBSRC

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Clinical Endocrinology Trust Visiting Professor Lecture

PL8

Abstract unavailable.

DOI: 10.1530/endoabs.31.PL8

Symposia

Irn bru, to drink or not to drink: endocrinology and iron

S1.1

Iron homeostasis: who are the major players?

Robert Evans
Brunel University, Uxbridge, Middlesex, UK.

Iron, an essential trace element for almost all organisms, has a structural or functional role in a number of proteins and enzymes. Total body iron amounts to ~35 and 45 mg/kg of body weight in healthy adult women and men, respectively. This iron is highly conserved and daily iron losses, normally only 0.5 to 2 mg via non-specific processes, are compensated for by absorption of an equivalent amount of iron from the diet. The precise regulation of cellular iron uptake and storage is very important if individuals are to avoid conditions of iron deficiency, as a result of a failure to absorb sufficient dietary iron, or iron overload, as a result of increased absorption of dietary iron or repeated blood transfusions, as in the case of patients with β-thalassaemia. Dietary iron and iron released from sites of haemoglobin breakdown is sequestered by transferrin, the iron-transport protein in plasma, however, it is unclear whether iron is passed directly or indirectly to transferrin. The iron saturation of transferrin is normally in the range 25–40% but in conditions of iron overload, transferrin can become fully iron-saturated and potentially toxic non-transferrin-bound iron (NTBI) can then be detected in plasma at levels of up to 10 μM.

Until about 15 years ago our understanding of the processes of dietary iron uptake, transport of iron in the blood, cellular uptake of iron and intracellular storage of iron was the result of extensive studies on the structure and function of a relatively small number of proteins, namely transferrin, ferritin, the classical transferrin receptor and iron-regulatory proteins. With the application of molecular biological and genetic techniques to the processes of mammalian iron metabolism, many novel proteins and enzymes, including HFE, hepcidin, ferroportin, DMT1, Dcytb, hephaestin, HCP1 and transferrin receptor two, have been identified and shown to play a crucial role in normal iron metabolism.

DOI: 10.1530/endoabs.31.S1.1

S1.2

The iron-regulatory hormone hepcidin

Elizabeta Nemeth
UCLA, Los Angeles, CA, USA.

The hepatic peptide hormone hepcidin is the principal regulator of iron absorption and tissue iron distribution. Hepcidin circulates in blood plasma and acts at nanomolar concentrations by inducing degradation of its receptor, the cellular iron exporter ferroportin. Ferroportin exports iron into plasma from absorptive enterocytes, from macrophages that recycle the iron of senescent erythrocytes, and from hepatocytes that store iron. Therefore, hepcidin-mediated degradation of ferroportin results in decreased iron absorption in the duodenum, regulating total body iron, as iron losses from the body are normally very small. Hepcidin effect on macrophage ferroportin inhibits the large flux of recycled iron into plasma and decreases plasma iron concentration, as iron is consumed for erythropoiesis and other processes. Hepcidin therefore acts as an endocrine regulator of total body iron stores and plasma iron concentration.

The synthesis of hepcidin is transcriptionally regulated by iron, erythropoiesis and inflammation. Extracellular and intracellular iron concentrations increase hepcidin transcription through a mechanism dependent on the bone morphogenetic protein pathway. Increased iron requirements of erythroid precursors for hemoglobin synthesis cause hepcidin suppression by an unknown pathway. Hepcidin production is also increased by inflammation, primarily through IL6. Dysregulation of these mechanisms leads to aberrant hepcidin production and the development of iron disorders.

Increased hepcidin concentrations in plasma cause or contribute to the pathogenesis of iron-restricted anemias including anemias associated with inflammation (rheumatoid arthritis, inflammatory bowel disease, obesity), chronic kidney disease, some cancers and iron-refractory iron deficiency anemia. Hepcidin deficiency causes iron overload in hereditary hemochromatosis as well as iron-loading anemias such as beta-thalassemia. The hepcidin–ferroportin axis is the principal regulator of extracellular iron homeostasis in health and disease, and is a promising target for the diagnosis and treatment of iron disorders.

Declaration of interest

I am a co-founder and Chief Scientific Officer of Intrinsic LifeSciences, a biotech company developing hepcidin diagnostics. I am also a co-founder of Merganser Biotech, a biotech company developing hepcidin-targeted therapeutics.

Declaration of funding

This work was supported by grants from the National Institute of Health.

DOI: 10.1530/endoabs.31.S1.2

S1.3

The celtic disease – haemochromatosis: a disease of iron overload

Heinz Zoller & Melanie Schranz
Medical University of Innsbruck, Innsbruck, Austria.

In contrast to the conventional view of haemochromatosis as a monogenic disease with autosomal recessive inheritance, more recent evidence form genetic, epidemiological, cell biological and clinical studies, challenges this view. The concept that haemochromatosis is an endocrine disorder of mixed etiology embraces the poylgenic nature of the disease, the low penetrance and the similarities in phenotype of genetic and acquired forms of iron overload. Key to understanding haemochromatosis as an endocrine disorder is that iron overload is caused by a deficiency of the iron hormone 'hepcidin'.

Accordingly, haemochromatosis can be viewed the 'diabetes of iron metabolism', where hepcidin and iron have a functional relationship comparable to insulin and glucose. Studies in mice defective for the genes encoding the hemochromatosis protein HFE, transferrin receptor two, and bone morphogenic protein six provide first insight into the 'iron sensor', which signals directly to the hepcidin promoter and thus control its transcription rate. Studies on the iron export protein ferroportin and its direct negative regulator hepcidin have shown that plasma iron is regulated via controlled release of iron from recycling macrophages, duodenal enterocytes and hepatocytes. This closes a feedback-loop, where hepcidin tightly controls plasma iron for sufficient delivery to cells utilizing the metal, while avoiding oxidative stress though uncontrolled release of iron into the circulation. In patients with haemochromatosis, hepcidin production is inappropriately low which causes uncontrolled release of iron into the circulation that results in increased transferrin saturation, which is the principal clinical biochemical defect in hemochromatosis. Genetically hemochromatosis is associated with homozygosity for the common C282Y polymorphism of the HFE gene. Although the exact function of this protein is still unknown, studies in cell and animal models of the disease suggest that HFE is part of the 'iron sensor' expressed in hepatocytes. Distribution of iron overload in HFE associated hemochromatosis is similar to other genetic iron overload disorders such as haemochromatosos associated with mutations in the transferrin receptor two gene, hemojuvelin gene or the hepcidin gene, which supports the role of these gene products in the iron sensing mechanism, that is being unravelled.

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Hormone maketh Man

S2.1

Anti-Müllerian hormone: a Sertoli cell hormone that can be used as a predictor of male hypogonadism

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Sertoli cells are the most active cell population in the prepubertal testis. During infancy and childhood, male hypogonadism can be evidenced by assessing Sertoli cell function without the need for stimulation tests. Anti-Müllerian hormone (AMH) is a distinctive serum marker of the prepubertal Sertoli cell, which is high from foetal life until puberty. AMH production is stimulated by FSH and potently inhibited by androgens. Initially used only to distinguish between patients with Persistent Müllerian duct syndrome (PMDS) due to AMH gene mutations and those with AMH receptor mutations, AMH diagnostic usefulness has extended to patients with other forms of disorders of sex development (DSD) and prepubertal male hypogonadism more generally. In boys with nonpalpable gonads, AMH is undetectable in anorchid patients, but detectable in those with abdominal testes. In prepubertal males with foetal- or childhood-onset primary or central hypogonadism affecting the whole testis (Sertoli + Leydig cells), serum AMH is low. Conversely, when hypogonadism only affects Leydig cells, serum AMH is normal/high. AMH is also normal/high in patients with androgen insensitivity. In patients of pubertal age with central hypogonadism, AMH is low for Tanner stage – reflecting lack of FSH stimulus –, but high for age – reflecting lack of testosterone inhibitory effect. FSH treatment results in serum AMH rise, whereas hCG treatment increases testosterone levels which inhibit AMH production. In summary, serum AMH determination is helpful in assessing gonadal function, without need for stimulation tests, and orientates the aetiological diagnosis of paediatric male hypogonadism. Furthermore, serum AMH is an excellent marker of FSH and androgen action in the testis.

Declaration of interest

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S2.2

The role of IGFs and the Sertoli cell in driving 'maleness'
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The way to maleness is a long process starting with fertilization when sperm delivers the testis-determining Y chromosome to the oocyte and ending with puberty and the action of testicular hormones. Since Sertoli cells are at the crossroads of the entire process, the analysis of the factors driving their differentiation and function is essential to the global understanding of male sexual development. By using mouse functional genetics, we will show that growth factors of the insulin/IGF family are required to mediate different aspects of gonadal development including Sertoli cell differentiation and function. Constitutive ablation of insulin/IGF signaling pathway led to defects in sex determination including absence Sertoli cell commitment and testicular differentiation as well as a delay in ovarian differentiation. In addition, we also show that the growth factors of the insulin family are the major drivers regulating the final number of Sertoli cells, testis size and daily sperm output in mice. These findings shed light on a crucial – but so far underestimated – signaling pathway underlying male sexual development in mice and potentially disorders of sex development (DSD) in humans.
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S2.3

Androgens and male fertility: a long way from the black box theory
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In males androgens are primarily made by testicular Leydig cells and act as essential regulators of both fetal masculinization and adult reproductive function. The impact of androgens on gene transcription is largely mediated by the androgen receptor (AR), a member of the steroid hormone super-family of ligand activated transcription factors. AR is expressed widely throughout the body, including several key somatic cell-types in the testis. Although we have known for many years that androgens are important regulators of testicular development and function, until recently it has been impossible to determine the specific roles androgens play in each cell-type, and how these cells respond to androgens to ensure correct male development and fertility. We have exploited conditional gene-targeting of AR using the Cre/lox system to ablate AR function in several key cell-types of the testis, including the Sertoli cells (SC), peritubular myoid cells (PTM), vascular smooth muscle cells (VSM), vascular endothelial cells (VE), and Leydig cells (LC); with a view to elucidating the cell-specific roles of androgen-signalling within the testis. These studies have identified novel roles for each cell-type in the promotion of male reproductive function. AR-signalling in SCs controls post-meiotic germ cell development and LC number. AR-signalling in PTM cells controls all stages of GC development, SC function and LC differentiation. Whilst AR-signalling in VE cells appears dispensable for testicular function, AR-signalling in VSM cells controls testicular blood-flow and LC function. Recent unpublished data suggests AR-signalling in LCs is also important for testicular function, acting via a novel mechanism. Taken together, these studies provide increasing evidence for the presence of a complex androgen-dependent paracrine signalling pathway within the testis, with each AR-expressing cell-type influencing others to ensure their correct development and function.
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Nurture not nature: epigenetics and disease susceptibility

S3.1

A mouse model of non-genetic inter-generational effects
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Non-genetic inheritance allows the environmental history of an individual to influence the next generation. This form of inheritance is well documented in worms, fruit-flies and plants. Observational evidence in mammals suggests two forms of non-genetic transgenerational effects; 'developmental programming' in response to early life exposures and germ-line transmission of an environmentally induced change i.e. 'epigenetic inheritance'. However, at present the mechanistic basis of these phenomena in mammals remains mysterious.
We have been studying a mammalian model encompassing both of these forms of inheritance. Inbred, C57BL/6J female mice are fed either a protein restricted or standard diet through gestation/lactation. The F1 offspring represent 'developmentally programmed' individuals. F1 males are maintained on control diet post-weaning and then mated to produce F2 offspring; our model for germ-line 'epigenetic inheritance'.
Previously we found that both F1 and F2 adults show both phenotypic and molecular changes. However, it is likely that many of these observations relate to downstream effects. What then, is the primary cause of this altered developmental trajectory? Our current work aims to define the primary phenotype in our model of mammalian epigenetic inheritance and correlate this with epigenetic perturbations in the male germ-line. To this end, transcriptomic analyses are being performed on F2 animals at multiple stages of development; prior to the first embryonic cellular differentiation, late gestation and as adults. Our recent findings will be discussed.
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S3.2

Nutritional programming of epigenetics in metabolic syndrome
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There is clear evidence from epidemiological studies that adverse conditions during pregnancy can programme changes in offspring which lead to increased risk of developing type two diabetes and obesity, two diseases associated with metabolic syndrome. Whilst earlier investigations focused on undernutrition in relation to famine, it is also relevant in the developed world to consider how dieting may programme lasting changes in the offspring.
In models of maternal undernutrition, the offspring have increased glucose intolerance and obesity in later life. Therefore our aims were to determine how hypothalamic genes critically involved in energy balance are affected by maternal undernutrition, both in the fetus and in adult offspring. We have utilised a sheep model, where hypothalamic maturation occurs prior to birth, on a similar trajectory to humans. In this model, ewes were moderately undernourished around the time of conception.
We found that maternal undernutrition is associated with epigenetic changes in the glucocorticoid receptor (GR) and concomitant increases in GR mRNA and protein in the fetal hypothalamus. These changes persisted in adult offspring studied up to five years after the maternal insult. The increases in GR expression were associated with an increased NPY mRNA and would predict the obese phenotype seen in adult male sheep. In contrast to the hypothalamus, different epigenetic changes in the GR were identified in the hippocampus and pituitary, but only in the adult offspring.
Therefore, adverse nutritional environments during pregnancy can programme epigenetic changes in the glucocorticoid receptor, resulting in changes in expression in areas of the brain responsible for feeding behaviour, energy expenditure and glucose homeostasis. That these epigenetic changes are identified in the fetus but persist in adult offspring provides a mechanism for the increased propensity for metabolic disease.
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S3.3

Epigenetic changes associated with prenatal exposure to famine in humans

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Epidemiologic studies suggest that adult disease risk may be associated with adverse environmental conditions early in development but the biological mechanisms behind these relations are unclear.

Our group used the circumstances of the Dutch famine of 1944–1945 to evaluate epigenetic changes in men and women with prenatal famine exposure during the Second World War. Study subjects were followed from birth to age ~58 years. To minimize potential confounding by maternal genes, early family environment or gender, all study subjects were paired to one unexposed same-sex sibling control. We examined 60 probands with exposure early in gestation and 62 with exposure late in gestation together with their unexposed sibling for a total of 122 pairs. We first established that DNA methylation at the IGF2 locus is associated with exposure in early gestation and that there may be sex-specific associations with DNA methylation at other loci.

In a randomly selected subgroup with exposure early in gestation ($n = 24$ pairs), we used next generation sequencing to systematically evaluate 28 classes of genomic regions within the genome for DNA methylation changes in relation to famine exposure. We found no associations with classes related to cancer prevalence such as CpG islands. We did however find associations with some classes defined by an open chromatin structure such as enhancers, especially those active during early blastocyst development. These associations were confirmed by mass spectrometry for four loci in this subgroup and also among the other 36 pairs with exposure early in gestation. The observed DNA methylation changes are in the order of 2–4%.

The identified loci map to genes involved in insulin signalling, regulation of developmental growth and lipid metabolism as will be presented in more detail. We are further exploring associations of the loci with measures of obesity and of glucose, insulin, and lipid metabolism.

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New Bone Biology – Is there life after RANK ligand?

S4.1

Abstract unavailable.

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S4.2

Inhibition of sclerostin in the treatment of osteoporosis

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During the past few years there have been important developments in the pharmacotherapy of osteoporosis. These developments were paralleled by significant progress in our understanding of the local regulation of bone metabolism. Studies of human and animal genetics led to identification of novel signaling pathways in bone cells, such as the Wnt signaling pathway, that provide targets for new bone building therapeutics for patients with osteoporosis. Fundamental for these developments have been studies of two rare bone sclerosing dysplasias, sclerosteosis and van Buchem disease, with closely related phenotypes characterized by bone overgrowth, which are due to defective production of sclerostin, a negative regulator of bone formation. The expression of sclerostin is restricted to osteocytes and is modified, among other, by mechanical loading and PTH. In addition, sclerostin stimulates bone resorption by a RANKL-mediated mechanism in osteocytes. An antibody to sclerostin given to OVX rats or intact monkeys increased bone formation at all bone envelopes without affecting, or even decreasing, bone resorption and improved bone strength. A single injection of an antibody to sclerostin to healthy postmenopausal women increased serum P1NP transiently decreased serum CTX and increased BMD after only 3 months. A recently reported phase II clinical study of monthly administration of a sclerostin antibody to postmenopausal women with low bone mass showed that this treatment increased BMD at all skeletal sites to levels higher than those attained with teriparatide and was well tolerated. Phase III studies are currently under way. The kinetics of bone remodeling in response to repeated administration of sclerostin inhibitors to humans need, however, to be clarified, particularly the nature and the magnitude of the response (transient or sustained). Apart from establishing the efficacy of these new molecules a critical issue for their introduction into clinical practice will be their tolerability and safety profile.

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S4.3

Parathyroid hormone-related protein as a potential treatment for osteoporosis

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Osteoporosis is defined as low bone mineral density and/or poor bone microarchitecture associated with increased risk of fractures. This chronic disease mainly affects postmenopausal women, but also older men, being increasingly considered as an age-related morbidity. Chronic glucocorticoid therapy and diabetes mellitus are also concomittant causes of osteopenia in aging subjects. The fact that osteoporosis-related fractures accompany the increased life span imposes a major challenge to our health systems. Skeletal alterations in osteoporosis are a consequence of an altered bone remodelling related to a decreased bone formation to bone resorption balance. Anticatabolic agents commonly used as osteoporosis therapies (namely, bisphosphonates) usually decrease bone formation due to their bone-remodelling inhibitory action. The first proven bone anabolic is parathyroid hormone (PTH) when administered intermittently, and as such is currently available for osteoporosis treatment. Its potential use to promote fracture healing and in tissue engineering applications is also being evaluated. PTH-related protein (PTHrP) is considered as a local PTH counterpart in bone. PTHrP within Its N-terminal region shows homology to PTH, justifying its interaction with the common PTH/PTHrP type 1 receptor (PPR) in osteoblasts, thereby modulating bone formation and bone turnover. Intermittent administration of N-terminal PTHrP peptides increases bone accrual to a similar extent to PTH in osteoporotic rodent models and in postmenopausal women. In contrast to PTH, however, bone anabolism of N-terminal PTHrP seems to occur without concomitant activation of bone resorption (thus avoiding the risk of hypercalcaemia). This interesting feature might be due to different interaction kinetics for PTH and PTHrP with the PPR. Furthermore, the PTH-unrelated C-terminal PTHrP domain exerts osteogenic actions, apparently associated with its anti-resorptive and anabolic properties, both *in vitro* and *in vivo*. Various promising options regarding the use of PTHrP-derived peptides to enhance bone accrual and bone repair will be discussed.

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S4.4

Abstract unavailable.

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Sex in the brain (Supported by *Endocrine Connections*)

S5.1

Abstract unavailable.

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S5.2

Abstract unavailable.

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S5.3

Abstract unavailable.

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S5.4

Hormone-dependent chromatin modifications regulating sexually differentiated animal behaviour

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Among all brain functions, the most strongly sexually differentiated are those which are directly related to reproduction. In addition to neuroendocrine controls of pituitary hormone release, we consider reproductive behaviors whose expression depends on steroid hormones. The hormone-dependent transcriptional activations in hypothalamic neurones long known to be required for female-specific reproductive behaviour, lordosis (Drive, MIT Press, 1999) involve binding to specific DNA response elements by the ligand-activated transcription factor Estrogen Receptor-alpha. Access to these DNA response elements is controlled by structural modifications of the N-termini of histones. I will summarize new data showing estrogen effects on histone chemistry in hypothalamic neurones that regulate lordosis behaviour. With chromatin immunoprecipitation (ChIP) we analyze sites on the promoters of the progesterone receptor and the oxytocin receptor genes, whose associated chromatin is modified by estrogen. As a side point, I will describe surprising relations between estrogenic and thyroid hormone effects on these promoters. In sum, we try to reason from detailed histone modifications in hypothalamic neurons to envision alterations of the defined lordosis behavior circuit, thus to explain the production of a biologically crucial behaviour.

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Making the glucocorticoid clock run smoothly (Supported by Addison's Disease self-help group)

S6.1

Monitoring glucocorticoid signaling and circadian clock function with transgenic zebrafish reporter lines

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Due to its rapid development and high fecundity, the zebrafish is a standard model in developmental biology and genetics. Furthermore, key hormone systems present in mammals are already active in zebrafish larvae. The endocrine system and the circadian clock are closely linked, and also the circadian system of zebrafish matures early in development. The small size of zebrafish larvae makes them particularly suitable for chemical screens *in vivo*: Larvae can be kept for days in 96 well plates and be exposed to different chemicals under varying environmental conditions. To harness these *in vivo* chemical screening possibilities of zebrafish larvae for chronobiology and endocrinology, we have generated transgenic zebrafish lines based on simple enhancer elements for the monitoring of circadian clock function and glucocorticoid signaling. Thus, a zebrafish line carrying a luciferase reporter construct regulated by 4 circadian E-box elements indicates core clock feedback loop activity early during development, and allows the detection of compound effects on period length over a broad range (1–12 h) *in vivo* under conditions suitable for high-throughput screening. Another luciferase reporter line carrying four glucocorticoid response elements (GREs) detects stress induced cortisol release in single larvae and can monitor the maturation of the stress response during development. This assay can also detect effects of environmental pollutants on endocrine signaling that are not detectable with cell culture assays: we observed a disruption of glucocorticoid signaling with environmentally relevant concentrations of an organotin compound that requires metabolization within the organism. A pilot screen with an FDA approved drug library of 640 compounds detected bona fide glucocorticoids present in the library with high specificity, as well as one additional compound stimulating cortisol production *in vivo*. Our lines provide versatile tools for chronobiology, stress research, environmental monitoring of endocrine disruptors and pharmaceutical screens targeting glucocorticoid signaling and circadian clock function.

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S6.2

Abstract unavailable.

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S6.3

Diurnal cortisol delivery: a novel tool for adrenal insufficiency

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Cortisol is an essential stress hormone and replacement with oral hydrocortisone is lifesaving in patients with adrenal insufficiency. Cortisol has a diurnal rhythm regulated by the central body clock and this rhythm is a metabolic signal for peripheral tissue clocks. Loss of cortisol rhythmicity is associated with fatigue, depression and insulin resistance. A general principle in endocrinology is to replace hormones to replicate physiological concentrations; however the pharmacokinetics of oral immediate release hydrocortisone make it impossible to fully mimic the cortisol rhythm and patients still have an increased morbidity and mortality despite replacement. Traditionally physicians have replaced hydrocortisone with a total daily dose based on the diurnal 24 h cortisol production rate with hydrocortisone given twice or thrice daily with the highest dose first thing in the morning. Monitoring treatment and dose titration has been much debated with some clinicians using cortisol day curves and others relying on clinical symptoms.

The main challenge being there is no established biomarker of cortisol activity. We have taken the view that an understanding of the cortisol circadian rhythm and hydrocortisone pharmacokinetics is essential when tailoring hydrocortisone dose. Using this approach we have developed a thrice daily, weight-related, dosing regimen and a pharmacokinetic and clinical method to monitoring treatment. However, this regimen still does not replicate the early morning rise in cortisol levels. To address this we have undertaken hydrocortisone infusion studies and developed a modified release formulation of hydrocortisone, Chronocort, that delivers the early morning rise in cortisol levels. We have undertaken pilot studies in patients with congenital adrenal hyperplasia to investigate the benefits of circadian cortisol therapy. Our argument for replicating the cortisol circadian rhythm is based on the observation that disruption of the rhythm is associated with ill health and preliminary data that circadian cortisol delivery improves disease control.

Declaration of interest

I am a Director of and hold equity in Diurnal Ltd.

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S6.4

Abstract unavailable.

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Thyroid hormone receptors – mutations and implications (Supported by *Journal of Molecular Endocrinology*)

S7.1

Physiologically distinct roles for thyroid hormone receptor isoforms

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The majority of T_3 actions are mediated by nuclear thyroid hormone receptors (TRα and TRβ), which act as hormone-inducible transcription factors. TRs are constitutively localised to the nucleus and, in the absence of hormone, bind to T_3-response elements (TREs) located in the promoter regions of T_3 target genes to mediate transcriptional repression. Entry of T_3 to the nucleus and high affinity binding to TRs results in de-repression of gene transcription and hormone-dependent activation of target gene expression. Several TRα and TRβ isoforms are transcribed from separate *THRA* and *THRB* genes. TRα1, TRβ1 and TRβ2 contain DNA and ligand-binding domains and act as fully functional T_3 receptors, whereas TRα2 lacks hormone-binding activity and acts as a weak antagonist *in vitro*, although its physiological function is unknown. TRα1 and TRβ1 are expressed widely but their relative levels differ during development and in adulthood due to tissue-specific and temporo-spatial regulation. Expression of TRβ2 is restricted to the hypothalamus and pituitary, where it mediates negative feedback regulation of TRH and TSH secretion, and to the cochlea and retina where it regulates timing of the onset of hearing and colour vision. Studies of mice with deletion or mutations of the *Thra* and *Thrb* genes have identified tissue-specific functions for TRα and TRβ. Thus, T_3 actions are mediated predominantly by TRα1 in the brain, heart, skeleton and gastro-intestinal tract and by TRβ1 in the hypothalamus, pituitary, liver and lung, whereas T_3 responses in other tissues such as skeletal muscle and adipose tissue are mediated by both TR isoforms. These studies have revealed the physiological complexity of thyroid hormone action, whilst characterisation of patients with resistance to thyroid hormone due to TR mutations has emphasised the translational importance of studies in genetically modified mice.

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S7.2

Human thyroid hormone receptor alpha mutations – a novel syndrome emerges

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Thyroid hormones act via receptor subtypes (TRα1, TRβ1, TRβ2) with differing, tissue-specific expression. We describe two unrelated cases of Resistance to Thyroid Hormone mediated by defective TRα1. Proband one (P1 female, age 6yrs) presented with lower segmental growth retardation (height < 10th centile), skeletal dysplasia (delayed bone age, femoral epiphyseal dysgenesis, delayed fusion of cranial sutures) and severe constipation. Proband two (P2, female, age 46yrs) also has disproportionate short stature (height <0.4th centile) and is dysmorphic (macrocephaly, coarse facies, multiple skin tags) with chronic constipation. Childhood cognitive impairment and epilepsy persist into adult life.

Many biochemical and physiological parameters were similar in P1 and P2: both exhibited low/low-normal FT4, high/high-normal FT3, low reverse T3 (rT3) and normal TSH levels; their resting heart and metabolic rates (BMR) were subnormal; circulating IGF1 levels were reduced; red blood cell mass was reduced with macrocytosis, but with normal haematinic and haemolytic indices.

Heterozygous mutations in *THRA*, resulting in carboxyterminally truncated TRα1 mutant proteins were identified in each subject ((P1: E403X; P2: fs388X). Both E403X and fs388X mutant TRα1 are transcriptionally inactive, fail to dissociate from corepressors, unable to recruit coactivators and inhibit wild type receptor action in a dominant-negative manner. T3-dependent target gene (KLF9) induction in peripheral blood mononuclear cells from patients is markedly attenuated.

Thyroxine treatment readily suppressed TSH and raised BMR and circulating IGF1 levels in both patients; serum SHBG levels rose and raised muscle CK normalised (P2); resting heart rates remained subnormal. General alertness, constipation and growth (P1) improved. These treatment responses reflect preserved hormone sensitivity in TRβ-expressing tissues (eg hypothalamus, pituitary, liver) and resistance in TRα-expressing tissues (e.g. myocardium).

This disorder exemplifies the existence of tissue-selective hypothyroidism, uncoupled from a dysregulated pituitary-thyroid axis, in humans.

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S7.3

Human thyroid hormone receptor β mutations-syndrome of resistance to thyroid hormone

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The classical form of thyroid hormone resistance (RTH) is characterized by elevated levels of circulating T_4 and T_3 in the presence of measurable serum TSH concentrations as a consequence of mutations of thyroid hormone β receptor (TRβ). RTH is a rare disorder, inherited in an autosomal dominant fashion. In the majority of the subjects, RTH is associated with heterozygous mutations in the TRβ gene. The mutant receptors display either reduced affinity for T_3 or impaired interaction with the cofactors, thus losing its ability to modulate target gene expression in different tissues. In contrast to what observed for other nuclear receptors, no mutations have been identified in the DNA-binding domain or in other regions of the receptor. To explain the presence of resistance in individuals heterozygous for the mutation, it was discovered that the mutant receptor exerts a dominant negative effect, which occurs because the mutant protein inhibits the activity of the wild type β- and α-receptors. In order to exert this effect, mutant receptors must retain normal dimerization and DNA binding properties. In about 10–15% of the cases with clinical and biochemical phenotype of RTH, no mutation could be found in the TRβ gene and this situation is defined as 'non-TRβ RTH'. It is speculated that these patients may have an abnormality of one of the cofactors or TH transporters into the cells. Heterozygous mutations in regions other than the three 'hot spots' may be clinically silent because lacking of dominant negative properties. RTH subjects are clinically defined as GRTH when display compensated hypothyroidism, or PRTH which exhibit variable symptoms of hyperthyroidism. There is a significant overlap between these two forms, being the symptoms variable. Differences in the degree of hormonal resistance are linked to the different levels of TRβ and TRα expression, in different tissues.

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S7.4

Nuclear receptor corepressors confer the actions of mutant thyroid hormone receptor α

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Patients with mutations of the thyroid hormone receptor α (*THRA*) gene display classic features of hypothyroidism with growth and developmental retardation,

skeletal dysplasia, and severe constipation, but with only borderline-abnormal thyroid hormone levels. These patients are heterozygotes, indicating that TRα1 mutants act in a dominant negative manner to mediate the clinical manifestations in these patients. However, the molecular mechanisms by which these TRα1 mutants act *in vivo* in a dominant negative fashion are not known. We tested the hypothesis that the severe hypothyroidism in patients with *THRA* mutations results from an inability of TRα1 mutants to properly release the nuclear corepressors (NCORs), thereby inhibiting thyroid hormone (T_3)-mediated transcription activity. We crossed $Thra1^{PV}$ mice expressing a dominant negative TRα1 mutant (TRα1PV) with mice expressing a mutant *Ncor1* allele ($Ncor1^{\Delta ID}$ mice). TRα1PV has the same mutated C-terminal sequence (-TLPRGL) with truncated termination at amino acid L406 as did two patients with frameshift mutations of the *THRA* gene. The NCOR1ΔID protein globally expressed by the mutant *Ncor1* allele cannot recruit the TR or PV mutant. Remarkably, the expression of NCOR1ΔID ameliorated abnormalities in the pituitary-thyroid axis of $Thra1^{PV/+}$ mice. The severe retarded growth, infertility, and delayed bone development were partially reverted in $Thra1^{PV/+}$ mice expressing NCOR1ΔID. The impaired adipogenesis was partially corrected by de-repression of peroxisome-proliferator activated receptor γ and CCAAT/enhancer-binding protein α genes, due to the inability of TRα1PV to recruit NCOR1ΔID. These *in vivo* data suggest that the aberrant recruitment of NCOR1 by TRα1 mutants could lead to clinical hypothyroidism in humans. Therefore, therapies aimed at the TRα1-NCOR1 interaction or its downstream actions could be tested as potential targets in treating TRα1 mutant-mediated hypothyroidism in patients.

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Non functioning pituitary tumours (Supported by *Endocrine-Related Cancer* and the Pituitary foundation

S8.1

Epidemiology and natural history of pituitary tumours

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Non-functioning pituitary adenomas (NFAs) are benign pituitary neoplasms arising from the adenohypophyseal cells. They are not associated with clinical evidence of hormonal hypersecretion and have a prevalence of 22 cases/100 000 people. They are diagnosed more commonly in males (2/3 of the NFA cases) and based on a recent UK community-based cross-sectional study, the median age at their diagnosis is 52 years (males 51 and females 43). At presentation, the majority is macroadenomas and their clinical manifestations are the result of pressure effects to surrounding structures.

Studies assessing the natural history of presumed, non-operated NFAs have shown probability of enlargement 19% for microadenomas and 44% for macroadenomas at 48 months follow-up. It has also been proposed that the event rate for growth per 100 patient-years is 12.53 (95% CI: 7.86–17.20) for macroadenomas and 3.32 (95% CI: 2.13–4.50) for microadenomas, whereas the risk of apoplexy is low. Factors predicting the behaviour of this group of NFAs are not clear.

Surgery remains the main management option for patients with macroadenomas exerting pressure effects to vital structures. Relapse rates in those treated only by surgery range between 6–46% (the risk is higher if there is extrasellar tumour remnant) and in those managed by surgery and adjuvant radiotherapy between 0–36%. Up to 20% of the relapses have been detected 10 years post-operatively necessitating long-term surveillance. Careful monitoring is also required for patients who had surgery following apoplexy of their NFA, as the risk of regrowth is not minimal (13% at 60 months). Reliable markers of tumour relapse at a pathological and/or molecular level are currently lacking.

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S8.2

Abstract unavailable.

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S8.3

Pathological markers of aggressive pituitary tumour behaviour

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Recent advances in molecular pathology have improved our knowledge on the pathogenesis of pituitary tumors, as well as on their growth potential, likelihood of recurrence, and prognosis. The development of reliable and prognostically informative methods of assessing tumor behavior is particularly important in pituitary tumors, where no precise correlation exists between morphology and clinical aggressiveness. Specific morphologic features (macroscopic invasion of the perisellar tissues, number of mitoses, Ki-67 labelling index, p53 expression) may serve as predictive markers of tumor behavior.

Apoptosis and mitoses represent two adverse and asynchronous events, maintaining the optimal cell numbers, and, as well as cytogenetic analysis, may be useful in defining the biological aggressiveness of pituitary tumors. From the genetic point of view, MEN1 tumors seem more aggressive, invasive and resistant to treatment requiring a very careful long-life follow-up. Recently, several studies attempted to identify new molecular markers (i.e. cyclooxygenase-2, galectin-3, angiogenesis molecules, pituitary tumor-transforming gene), that need to be validated. Among these, several are represented by therapeutic targets of new (and old) molecularly targeted therapies. Immunohistochemical detection of somatostatin receptors is important, being their density directly related to the effectiveness of somatostatin analogues. Similarly, the outcome of treatment with temozolomide, an orally administered alkylating agent, has been related to the expression of *O*(6)-methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme. Studies involving cDNA microarrays, stem cells and microenvironment may reveal additional important information to identify predictive markers in the near future.

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S8.4

Aggressive pituitary tumours and temozolomide treatment

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Aggressive pituitary tumours are particularly challenging to clinicians in terms of diagnosis and treatment. They may first present as typical pituitary adenomas, with a delayed appearance of aggressive signs, or initially as aggressive tumours. Predicting pituitary tumour behaviour remains difficult: increased mitotic, Ki-67, and P53 indexes may be associated with tumour aggressiveness. True pituitary carcinomas are rare, representing about 0.2% of all pituitary tumours. The treatment of pituitary carcinomas and aggressive pituitary tumours includes surgery, adjuvant medical treatment, external beam radiotherapy, and chemotherapy. Until recently, the treatment of pituitary carcinomas was mainly palliative and did not seem to increase overall survival. Recent case reports detailed the successful use of temozolomide, an orally administered alkylating agent used to treat malignant gliomas, in the management of carcinomas and aggressive pituitary tumours. The outcome of treatment might depend on the expression of O^6-methylguanine-DNA methyltransferase (MGMT), a DNA repair enzyme that potentially interferes with drug efficacy. However, on an individual basis, the prognostic value of determining pre-treatment MGMT status does not seem to preclude a therapeutic attempt. Overall about 50–60% of such aggressive tumours respond to temozolomide.

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Novel aspects of GPCR signalling (Supported by the *Journal of Endocrinology*)

S9.1

GPCR mutations and reproduction

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The mechanisms controlling the timing of puberty remain largely unknown. Recent insights into genetic causes of pubertal disorders have provided important advances in our understanding of the physiology underlying this developmental process. Mutations in genes important in neuroendocrine pathways controlling GnRH release and LH and FSH secretion have been identified in patients with isolated hypogonadotropic hypogonadism, Kallmann syndrome, and central precocious puberty. Many of the genes implicated encode G protein-coupled receptors (GPCRs) and their cognate ligands, including: i) *KISS1/KISS1R,* encoding kisspeptin and its receptor (KISS1R), ii) *TAC3/TACR3*, encoding neurokinin B and the neurokinin 3 receptor (NK3R), iii) *PROK2/PROKR2*, encoding prokineticin 2 and prokineticin receptor 2 (PROKR2), and iv) *GNRH1/GNRHR*, encoding GnRH itself and its receptor, GnRHR. Mutations in these GPCRs have been described in both heterozygous and homozygous states in patients with varying degrees of GnRH dysregulation. These mutations have been identified in diverse functional domains of the receptors. Elucidation of structure-function relationships for these GPCRs and of the key mechanisms by which their activation mediates cellular and biological responses have become increasingly important for our understanding of the reproductive abnormalities resulting from mutations in these genes. The identification of the critical domains of these receptors important for their activity and of their downstream pathways of signaling will advance our understanding of the function of these receptors in the control of GnRH release.

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S9.2

Persistent signaling by TSH receptors

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Signaling by TSH receptor (TSHR) was thought to terminate after withdrawal of TSH. Recently, however, TSHR was found to signal persistently even after TSH withdrawal via both the cAMP and inositol-1,4,5-trisphosphate pathways. Similar persistent signaling was found for other G protein-coupled receptors, such as the parathyroid hormone receptor, which stimulates the cAMP pathway, and the S1P1 receptor, which inhibits the cAMP pathway. For TSHR, a controversy has developed as to whether persistent signaling is cell type-specific. We reported that persistent TSHR signaling occurs in a model cell system of HEK293 cells stably overexpressing human TSHRs whereas another group reported that persistent signaling was found in mouse thyroid follicles but not in HEK293 cells. If thyroid cells were different from other cells regarding persistent signaling, this would have important implications for TSHR biology. Specifically, as TSH is secreted in a pulsatile fashion, thyroid cells would respond persistently but other cells only transiently. I will discuss our new findings, which confirm our previous observations, that TSHR signals persistently in HEK293 cells (and in human thyrocytes).

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S9.3

***In vivo* dimerization of LH receptors**

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The classical model of GPCR activation entails the binding of a single ligand to a single receptor molecule, followed by transmembrane signal transduction to the appropriate G protein(s). The possibility of GPCRs functioning as dimers or oligomers still remains controversial, and is largely based on *in vitro* studies on transfected cells. The glycoprotein hormone receptors, including that of LH (LHCGR), differ from most GPCRs by their large extracellular ligand-binding domain distinct from the transmembrane signaling domain. Therefore, numerous LHCGR mutants are specifically devoid of either ligand binding or intracellular signaling. If such mutants are coexpressed in transfected cells, they can partially rescue ligand-evoked signaling, providing compelling evidence for functional complementation (or intermolecular co-operation) possibly through dimerization. We tested the physiological significance of this mode of receptor activation by co-expressing in BAC transgenic mice either a binding- or signaling deficient mutant of LHCGR, crossed into the LHCGR null background. Either of the mutant LHCGRs singularly expressed did not alter the hypogonadal phenotype of LHCGR knockout (KO) mice. However, when both mutants were coexpressed in the KO background, the eugonadal and fertile wild-type phenotype of male mice was restored. Interestingly, female mice co-expressing the two mutant receptors in the KO background remained hypogonadal and infertile. These findings indicate that binding- and signaling-deficient LHCGRs are able to partially recover the signaling that is sufficient for Leydig cell activation and fertility in male mice. Functional complementation in females is not sufficient to revert the hypogonadal KO phenotype, probably due to the higher receptor activation required (ovulatory LH peak), but it is also possible that only selected signaling pathways (biased agonism) are activated upon LHCGR functional complementation.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.
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S9.4

Allosteric LH, TSH and FSH receptor signaling

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The LH receptor (LHR) together with the FSH receptor (FSHR) and TSH receptor (TSHR) constitute a highly conserved subgroup of G protein-coupled receptors. Activation of these receptors requires the binding of the glycoprotein hormones to the long and divergent N-terminus of the receptor and the intramolecular signal transduction from the hormone-receptor complex to the transmembrane domain of the receptor. The main signaling pathway of the LHR, TSHR and FSHR is stimulation of adenylyl cyclase via Gs proteins but they may couple to phospholipase C via Gq proteins as well. Interaction of activated receptors with β-arrestin has also been demonstrated but this coupling mechanism has been studied in much less detail.

Research and development within various pharmaceutical companies have been focused on developing low molecular weight (LMW) agonists and antagonists for these receptors. Such LMW allosteric receptor modulators offer increased homogeneity and consistency, better compound stability compared to glycoprotein hormones and, preferably can be administered orally to improve patient convenience and compliance. At MSD (formerly Organon), allosteric nanomolar potent and orally active LMW agonists and antagonists of the LHR, TSHR and FSHR have been developed. These compounds have been shown to interact with the transmembrane domains instead of the N-terminus of the receptor.

In this presentation, the allosteric profile of two nanomolar potent, orally active LHR and FSHR agonists (Org 42599 and Org 214444-0 respectively), and one TSHR antagonist (Org 274179-0) will be presented. Highlighted will be i) biased signaling and rescue of expression and signaling of intracellularly retained mutant receptors identified in human patients with impaired reproductive function (Org 42599), ii) full inhibition of receptor signaling without affecting hormone binding (Org 274179-0) and iii) enhancement of binding and signaling of the glycoprotein hormone (Org 214444-0).

Declaration of interest
The presenting author (CVK) has been employee of MSD (Oss, The Netherlands).
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Lipodystrophy – The perils of being thin

S10.1

Abstract unavailable.

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S10.2

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S10.3

HIV lipodystrophy

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HIV-1-infected patients under antirretroviral treatment develop the so-called 'lipodystrophy syndrome'. It is characterized by peripheral lipoatrophy (face, limbs), visceral lipohypertrophy, and, sometimes abnormal accumulation of subcutaneous fat in specific sites, especially the dorso-cervical area. The abnormal fat distribution is associated with systemic alterations characteristic of the metabolic syndrome (dyslipidemia, insulin resistance). Drugs are importantly involved in the development of the syndrome, but studies of fat biopsies from HIV-1-infected patients that had not yet started treatment revealed that alterations in adipose tissue are already present in association with the infection. A major alteration in the patients is mitochondrial toxicity, evidenced by mitochondrial DNA depletion in fat. This is mainly due to some of the antiretroviral drugs. However, the mitochondrial DNA depletion found commonly in atrophic and hypertrophic areas makes unlikely that this alteration was responsible for the opposite behavior of fat at distinct anatomical sites. Enhanced expression of pro-inflammatory cytokines (TNFα, MCP-1) takes place in lipoatrophic areas, whereas, in hypertrophic sites, this alteration is either non-present (dorso-cervical lipomatosis) or it has a minor intensity (visceral fat). The pro-inflammatory status in subcutaneous areas prone to atrophy is associated with a repression of the expression of adipogenic genes, such as PPARgamma, and its metabolic target genes (GLUT4, lipoprotein lipase). This alteration in adipogenesis is absent in hypertrophic areas. It is likely that the pro-inflammatory induction was a first event in eliciting the anti-adipogenic profile in atrophic fat. Finally, the dorso-cervical hypertrophic areas have features reminiscent of lipomatosis (enhanced proliferation, lowered telomere length) and a distorted brown-to-white adipose tissue phenotype (expression of the brown fat marker gene UCP1). HIV-1 lipodsytrophy appears as a multifaceted alteration in which inflammatory and adipogenic alterations act locally in a differential manner, with overall systemic alterations being associated with the complex distribution of fat disturbances.

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S10.4

Approach to lipodystrophy management: a new national service

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Lipodystrophy is a group of rare disorders characterized by complete or partial loss of adipose tissue. Lipodystrophy can be inherited or acquired and may present in childhood or in adults. Metabolic sequelae of lipodystrophy include severe insulin resistance (SIR), type 2 diabetes, hypertriglyceridaemia, hepatic steatosis and hyperandrogenism. Research in our centre has produced a database of several hundred patients with lipodystrophy and/or SIR and has contributed to identification of several novel genetic syndromes of SIR. We have also recently established integrated biochemical and genetic diagnostic algorithms for patients with SIR. In April 2011 this was recognised through commissioning of a national multidisciplinary NHS service for patients from England with lipodystrophy and/or SIR by the National Specialist Commissioning Team (NSCT). The National SIR Service aims to provide diagnostic, therapeutic and educational support for patients with lipodystrophy and/or SIR, and to establish and disseminate evidence-based recommendations for their clinical management. The service is based at Addenbrooke's Hospital, Cambridge. We review patients at a weekly multidisciplinary clinic with a Consultant Physician, Consultant Paediatrician, Specialist Diabetes Nurse and Dietitian. Individualised dietary management is a key component. For selected patients treatment with agents such as leptin, GLP-1 agonists, Humulin R U500 insulin and rhIGF1 is supervised directly. Close contact is maintained with local specialists and primary carers. The service accepts referral of patients from England with one) Clinically diagnosed lipodystrophy (generalised or partial), two) Clinical suspicion of insulin receptor defects, from neonatal Donohue Syndrome to adult type A insulin resistance and three) Unexplained SIR with BMI $<30\ kg/m^2$ and acanthosis nigricans and/or severe hyperinsulinaemia. In conclusion, the National Severe Insulin Resistance Service is a new NSCT-funded multidisciplinary service aiming to improve management and clinical outcomes for patients with lipodystrophy and/or SIR. Current management strategies for patients with lipodystrophy will be discussed.

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Guts, brains and bariatric surgery

S11.1

Abstract unavailable.

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S11.2

Ghrelin, a gut-brain signal of importance for food reward

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Accumulating evidence suggests that ghrelin's physiological role extends beyond appetite and energy balance to include reward-seeking behaviour both for food (a natural reward) and chemical drugs. The neurochemical circuitry that links ghrelin to reward behavior and the level of the mesolimbic reward system remains unclear. Ghrelin receptors can be found on the ventral tegmental area (VTA) dopamine neurons. It is not known, however, which dopaminergic projections are relevant for ghrelin's effects on reward, since VTA dopamine neurons send projections to several brain areas relevant for reward behavior including the nucleus accumbens (NAc), amygdala and prefrontal cortex. We found that food-motivated behaviour assessed in the progressive ratio lever-pressing for sucrose paradigm could be driven from the VTA but not the NAc. Interestingly, pretreatment with either a D1-like or D2 receptor antagonist into the NAc completely blocked the food reward effect of VTA ghrelin, leaving chow intake intact. This suggests a role for the VTA-NAc dopaminergic signaling in food

reward behavior but not for food intake. The idea that ghrelin's effects on food intake and food reward behavior engage different reward circuitry is also supported by studies in which we were able to parse ghrelin's effects these behaviors pharmacologically using mu-preferring opioid receptor antagonist or an NPY Y1 receptor antagonist. Supported by EC: FP7-HEALTH-2009-241592; FP7-KBBE-2009-3-245009.

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S11.3

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S11.4

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Thymic function and autoimmune endocrine disease

S12.1

The thymus medulla, aire and autoimmunity

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A key role of the thymic medulla is to negatively select $CD4^+$ and $CD8^+$ thymocytes expressing potentially autoreactive αβT-cell receptors (αβTCR), a process important for T-cell tolerance induction. It is known that tolerance induction in the thymus involves multiple processes, and the thymus medulla is also known to contribute through guiding the generation and selection of natural $FoxP3^+$ regulatory T-cells (nT-Reg). Of the cells contained within the medulla, thymic medullary thymic epithelial cells (mTEC) have been implicated as a key regulator of the major aspects of tolerance induction. This is at least in part through their expression of the autoimmune regulator (AIRE) gene, the only known regulator of intrathymic peripheral tissue antigen (PTA) expression, and the gene mutated in autoimmune polyendocrinopathy (APECED). Importantly, the role of the $Aire^+$ mTEC subset in T-cell tolerance mechanisms is poorly understood. Here we have investigated the cellular and molecular processes that lead to the formation of $Aire^+$ mTEC, and investigated their role in T-cell tolerance induction. We provide evidence that the establishment of tolerogenic mTEC in the fetal and neonatal periods is under the control of cellular components of the innate immune system, including invariant gamma delta T-cells, and lymphoid tissue inducer cells. Thus, during a time window that is known to be essential for tolerance induction, the innate immune system acts to establish Aire-expressing tolerogenic thymic microenvironments that ultimately shape the nascent αβTCR repertoire. Moreover, by performing in vivo thymus transplantation experiments involving thymic tissue genetically deficient in mTEC, we provide evidence that the thymus medulla controls $FoxP3^+$T-Reg development through the generation of their $FoxP3^-CD25^+$ precursors. Collectively, our data shows that medullary thymic microenvironments that are shaped by the innate immune system play an essential role in imposing tolerance on the emerging adaptive immune system through both central and peripheral tolerance mechanisms.

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S12.2

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S12.3

Thymic microenvironments for T cell repertoire formation

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During the development in the thymus, a virgin repertoire of diverse TCR-αβ recognition specificities in immature T cells is selected through positive and negative selection to form a functionally competent and self-tolerant repertoire of mature T cells. Positive selection supports the survival of self-MHC-restricted thymocytes that receive low-affinity TCR engagement, whereas negative selection deletes potentially harmful self-reactive thymocytes upon high-affinity TCR engagement. Recent advances in the biology of thymic stromal cells have indicated that proximal interplays among developing T cells, dendritic cells, and thymic medullary epithelial cells that promiscuously express tissue-specific self-antigens is essential for the establishment of a self-tolerant TCR repertoire. It has also been indicated that the formation of an immunocompetent TCR repertoire requires positive selection by thymic cortical epithelial cells that express unique protein degradation machineries, including the β5t-containing thymoproteasome. These results suggest an essential role of self-peptide repertoires specifically expressed by multiple thymic microenvironments in the development of an immunocompetent and self-tolerant T cell repertoire.

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S12.4

Regulatory T cells, CTLA-4 and autoimmune disease

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The T cell immune system exists in a state of balance, poised to react to invading pathogens but at the same time constantly being restrained from attacking our own tissues. Several strategies are employed in order to minimise our own self-reactivity. First amongst these processes is the deletion of T cells in the thymus, however this process is incomplete and self-reactive T cells still populate our immune systems. A second layer of control is exerted by regulatory T cells (Treg) which act to restrain self-reactivity by dominantly suppressing T cell responses. As expected, deficiency in Treg results in profound auto- immune dysregulation polyendocrinopathy and enteropathy X-linked syndrome(IPEX). How Treg function to prevent autoimmunity is therefore of considerable interest. The protein CTLA-4 is highly expressed on Treg and is also associated with a number of autoimmune diseases in genome wide studies. We have recently identified a novel molecular basis for CTLA-4 function where CTLA-4 acts as a molecular 'hoover' removing stimulatory ligands from antigen presenting cells (1). This talk will discuss the impact of CTLA-4 on regulatory T cell function along with strategies for enhancing regulatory T cell control of responses relevant to autoimmune disease.

Reference
1. Qureshi OS, et al. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science* 2011; **332** (6029): 600–3.

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Clinical Management Workshops

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Management controversies in parathyroid disease

CMW1.1

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CMW1.2

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CMW1.4

Medical management of primary hyperparathyroidism

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Surgical parathyroidectomy remains the most cost effective treatment for primary hyperparathyroidism but where surgery is inappropriate because of significant co-morbidity there is a place for medical therapy. Calcimimetics are type II agonists (requiring calcium for activity) of the calcium sensing receptor (CaSR). The first compound of this class is cinacalcet which reduces PTH by about 50% at 4 h with recovery to within 20% of baseline over the next 8 h. Despite these fluctuations in PTH it is possible to achieve stable normocalcaemia over a 24 h period without loss of effect in studies extending out to 4 years. Most patients with modest HPTH (<2.85 mmol/l) will require 60–90 mg/day although those with hypercalcaemia $>$ 3 mmol/l will require 90 mg twice daily or more to achieve normocalcaemia.

Despite the achievement of normocalcaemia calcium excretion does not fall because the reduced filtered load of calcium ($Ca^{++} \times$ GFR) is balanced by loss of distal renal tubular calcium reabsorption consequent on the fall in PTH. Serum concentrations of 1,25 $(OH)_2$ D are also maintained because a reduction due to the fall in PTH is balanced by direct stimulation of theproximal convoluted tubular CaSR which activates the 1 α hydroxylase. A disappointing finding is that bone turnover and BMD are notimproved by cinacalcet. This may be due to the transientnature of the control of PTH since BMD increases when PTH is reduced by parathyroidectomy. It is also likely that the changes in PTH produced by cinacalcet have a more prolonged time course than, for example, the short spike of concentration produced by teriparatide which is anabolic to bone. However several studies have shown that bisphosphonates protect the skeleton from HPTH and in patients with osteoporosis due to HPTH co-prescription with cinacalcet will be needed.

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How Do I Do It?

CMW2.1

Abstract unavailable.

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CMW2.2

How do I investigate and manage hypomagnesaemia?

John Ayuk
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Serum magnesium concentration is regulated by the balance between intestinal absorption and renal excretion. Hypomagnesaemia is relatively common, with an estimated prevalence in the general population ranging from 2.5 to 15%. It may result from inadequate magnesium intake, increased gastrointestinal or renal loss, or redistribution from extracellular to intracellular space. Drug-induced hypomagnesaemia, particularly related to proton-pump inhibitor (PPI) therapy, is being increasingly recognised. Although most patients with hypomagnesaemia are asymptomatic, manifestations may include neuromuscular, cardiovascular and metabolic features.

Measurement of total serum magnesium is the method of choice for determining clinical magnesium status. However serum magnesium may not always accurately reflect the intracellular magnesium status and a subject with normal serum magnesium levels may have total body magnesium depletion. Although 30% of serum magnesium is bound to albumin and is therefore inactive, conventionally serum magnesium concentrations are not 'adjusted' for albumin concentrations, as there is generally a high correlation between serum total and ionised magnesium concentrations.

Once hypomagnesaemia is confirmed, in many cases the cause can be obtained from the history. If no cause is apparent, the distinction between gastrointestinal and renal losses can be made by measuring 24-h urinary magnesium excretion or fractional excretion of magnesium.

Patients with symptomatic hypomagnesaemia should be treated with intravenous magnesium, reserving oral replacement for asymptomatic patients. Consensus statements suggest administration of 8–12 g of magnesium sulphate in the first 24 h followed by 4–6 g/day for 3 or 4 days to replete body stores. Oral magnesium salts can be used to supplement body magnesium, but they are generally not well absorbed from the gastro-intestinal tract.

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CMW2.3

How do I monitor and follow up transgender patients using hormonal therapies?

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Gender identity disorder is not a rare condition occurring in 1:7440 born male and 1:31 153 born female individuals. Although gender transition is supervised in specialist clinics, post transition the patients are discharged back to primary or secondary care follow up. In my session I will discuss the process of gender transition, common hormonal regimens used and their monitoring. I will also discuss safety studies and the monitoring of long term follow up of individuals taking cross sex hormone therapy.

For transmen standard hormone replacement doses of androgen appear to be adequate for general long term health. Although cardiovascular risk in transmen is increased compared to the female population the myocardial infarction risk is still only one third the general male population.

In transwomen oestrogen doses of up to 5-times standard hormone replacing doses are used. In transwomen the risk of DVT is 20-fold the general population, however it appears that they oestrogen type used is important in this risk and modern regimens using oestradiol are much safer than older regimens using premarin. Similarly recent studies suggest that the use of ethinyl oestradiol is associated with and increased risk of cardiovascular events compared with other forms of oestrogen and has implications for the long term management of oestrogen replacement. The breast cancer risk of transwomen is however

minimal. The studies support the use of oestradiol as the safest form of oestrogen in transwomen.
Overall the long term safety of hormone replacement in trans-patients is safe with a standard mortality ratio of 1 compared with the general population.
Declaration of interest
I fully declare a conflict of interest. I have performed paid lectures for Bayer and Pfiser.

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CMW2.4

How do I manage the pregnant patient with a prolactinoma?

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There are two issues: i) dopamine agonist (DA) safety for mother and baby, and ii) risk of oestrogen-induced prolactinoma enlargement. Bromocriptine (BC) and Cabergoline (CAB) are both safe for ovulation induction but the safety database is larger for BC (6239 pregnancies) than for CAB (789). Neither drug causes increases in miscarriage, premature delivery, multiple births or congenital malformations, compared to data for normal pregnancy. Risk of symptomatic tumour enlargement during pregnancy depends on prolactinoma size and its responsiveness to DA therapy before pregnancy. For micro prolactinoma the risk is only 2%. For untreated macro prolactinoma, the risk is ~20% but the figure is probably ~10% for DA-responsive tumours treated medically for at least 6 months before conception. DA-induced tumour fibrosis may limit early reexpansion.
Management
For patients with micro prolactinoma, DA can be safely stopped when pregnancy is confirmed. Formal visual fields and serum PRL measurements are unnecessary. Breast feeding can be encouraged and up to one-third of tumours remit after pregnancy. For patients with macro prolactinoma, DA should be used for 6–12 months before conception is attempted. If post-treatment MRI shows *intra*sellar tumour, DA can be stopped when pregnancy is confirmed; fewer than 10% develop symptomatic enlargement (for which DA therapy can be restarted, usually with BC). If significant *extra*sellar tumour persists, either debulking surgery before pregnancy or continued DA therapy throughout pregnancy can be considered. The safety data for BC and CAB usage throughout pregnancy are broadly reassuring but experience remains limited. Visual fields should be monitored every 1–3 m and MRI reserved for those with symptoms of tumour enlargement.
Recommended reading
Lebbe M *et al*, Clin Endocrinol (2010), **73**:236 (outcome of 100 cabergoline-initiated pregnancies). Molitch ME. Prolactinoma in pregnancy. *Best Pract Res Clin Endocrinol Metab* (2011), **25**:885 (comprehensive review).

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CMW2.5

How and when do I induce puberty in males?

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Induction of puberty may be required in boys, if puberty is either delayed or arrested. Boys with delayed puberty are often significantly concerned about their physical immaturity, short stature and perceived difference in appearance from peers. The commonest cause of delayed puberty is constitutional delay of growth and puberty (CDGP), a relatively benign condition. However, hypergonadotrophic hypogonadism due to Klinefelter syndrome and hypogonadotrophic hypogonadism (HH) due to brain tumours and Kallmann syndrome, should also be considered in the differential diagnoses. In boys with CDGP, induction of puberty may be beneficial to improve height velocity and pubertal progress, and hence allay anxiety. In children with chronic illnesses causing delayed puberty, induction of puberty may be beneficial to optimise bone mineralisation. Several androgen preparations, administered by varying routes and regimens have been used for the induction of puberty; a short course of injectable testosterone remains the commonest method, with satisfactory puberty and height outcomes achieved in most boys with CDGP. Long acting depot preparations of testosterone may be beneficial in young adults with persistently delayed puberty with likely HH, although injectable gonadotrophin induction of puberty may also be considered.

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CMW2.6

Abstract unavailable.

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PCOS – why, how and what

CMW3.1

Consensus on women's health aspects of PCOS

Adam Balen
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Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder present in 5–15% of women in the reproductive age group. There have been a number of international consensus meetings that have focussed on diagnosis and management, with the third ESHRE/ASRM sponsored PCOS consensus workshop in 2010 looking at the various health aspects of PCOS, apart from the well known effects on reproduction. These include problems during adolescence, hirsutism and acne, menstrual cycle abnormalities, quality of life, ethnicity, long-term metabolic and cardiovascular health and cancer risk. The endocrine features normally draw attention due to symptoms of irregular menstrual cycles, oligo or anovulation leading to delayed conception and hyperandrogenaemic features such as hirsutism and acne. Insulin resistance (IR) and resultant hyperinsulinaemia are considered to be the underlying pathophysiological features for many with PCOS. It is not easy to analyze the possible role of PCOS, independent from overweight, metabolic syndrome, insulin resistance and diabetes, on long-term health. In addition to the long-term metabolic and cardiovascular risks there is also an increased risk of endometrial cancer.

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CMW3.2

Abstract unavailable.

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CMW3.3

Is there a place for metformin in pcos

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Metformin has been used extensively in multiple reproductive settings including to ameliorate hyperandrogenism and chronic anovulation, to treat infertility, to prevent miscarriage and to prevent later pregnancy complications. Metformin does result in modest improvements in the PCOS phenotype with reductions in circulating insulin and testosterone levels, weight loss, and improved menstrual/ovulatory frequency. It is relatively ineffective as a solo agent to treat infertility, and further has a relative anti-fecundity compared to clomiphene alone, though it likely has a lower multiple pregnancy rate. Clomiphene remains the first choice for infertility therapy and the gold standard for women with PCOS. Metformin may have benefit as an adjuvant agent with clomiphene in select populations, such as obese women. Metformin may be useful to prevent OHSS

when used in conjunction with gonadotropins. The benefit of metformin in IVF *per se* is uncertain. The use of metformin to prevent pregnancy loss or to prevent pregnancy complications is still experimental and better trials are needed to guide therapy. Current level of evidence recommends caution in the extended use of metformin during pregnancy. Metformin has been shown to delay the progression to diabetes in high risk female populations, such as those with IGT. It may also have some benefit for the treatment of premature pubarche. Metformin is ineffective as a weight loss agent in women with PCOS. Criteria for stopping Metformin (other then pregnancy or unacceptable side effects) are uncertain, and the long-term risk benefits are unknown in women with PCOS.

Declaration of interest

I fully declare a conflict of interest.

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CMW3.4

Diagnosis and management of hirsutism

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Hirsutism is the presence of excessive terminal hair in androgen dependent areas of the female body. A frequent medical complaint that affects ~ 12% of Caucasian premenopausal women, hirsutism usually results from relatively benign functional disorders. Among them, the polycystic ovary syndrome is the most frequent etiology.

The most important tool for the diagnosis of hirsutism is a complete clinical history and physical examination. Functional causes begin peripubertally and progress slowly, whereas the very rare androgen-secreting neoplasms have a sudden onset and a rapid progression of hirsutism, and usually associate clinical signs of virilization and defeminization. The correct diagnostic approach to the hirsute patient requires, in all cases, quantification of hirsutism using the modified Ferriman–Gallwey score, measurement of circulating androgen concentrations, a detailed study of ovulatory function and, possibly, ultrasound assessment of polycystic ovarian morphology. Chronic management must consider not only amelioration of hirsutism but also treatment of the underlying etiology and of any metabolic associations. When caused by a functional disorder, treatment of hirsutism should be chronic and should include cosmetic as well as pharmacological interventions including oral contraceptives and/or antiandrogens. For non-functional disorders treatment should focus on solving the underlying etiology as hirsutism is usually responsive to the elimination of the source of androgen excess.

Declaration of funding

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Managing Hypoglycaemia

CMW4.1

Hypoglycaemia in diabetes: effects on cerebral and autonomic function

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Hypoglycaemia (low blood glucose) is the most important acute side effect of insulin and insulin secretagogue therapies for diabetes mellitus. The initial response to a threat to the circulating glucose concentration is cessation of endogenous insulin and stimulation of pancreatic glucagon release – neither of which happen in insulin deficient diabetes. Patients with diabetes depend on other autonomic and most importantly symptomatic responses to defend against falls in blood glucose low enough seriously to impair cognitive function. Up to 40% of people with established type 1 diabetes and an as-yet undetermined proportion of people with type 2 develop defects in these further responses which means that cortical impairment is the first clinical correlate of a falling glucose. This state increases risk of severe hypoglycaemia six fold. The defects in glucose counterregulation and hypoglycaemia perception are induced by antecedent exposure to hypoglycaemia and may be associated with other elements of autonomic dysfunction specific to the state of unawareness (i.e. not necessarily to classical diabetic autonomic neuropathy). Hypoglycaemia also affects brain function in the medium term, in that memory formation and consolidation are both adversely affected by low glucose, although in adults there is no strong evidence for permanent brain dysfunction from hypoglycaemic episodes from which apparently full recovery is made at the time.

The regional brain responses to acute symptomatic hypoglycaemia include stimulation of glucose-responsive neurones in the hypothalamus and brain stem nuclei and activation of brain regions involved in stress (the HPA axis); and interoception (the anterior cingulate cortex), as well as changes in activation in reward and appetite control pathways. Many of these central responses are altered in hypoglycaemia unaware patients. The differences in cortical responses may underlie clinical observations of low concern about hypoglycaemia expressed by many diabetic patients with hypoglycaemia unawareness and of reduced compliance with regimen changes intended to prevent hypoglycaemia. On-going research is using education and technology to help prevent recurrent hypoglycaemia in these patients and restore their protection from severe hypoglycaemia.

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The work to be presented is a review but the author's own work has received grant funding from Diabetes UK, Juvenile Diabetes Research Foundation International, Wellcome Trust, Medtronic Inc, The King's College Hospital Charity.

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CMW4.2

Abstract unavailable.

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CMW4.3

Hypoglycaemia in neonates and Children

Khalid Hussain
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Hypoglycaemia is one of the most common biochemical abnormalities observed in the neonatal, infancy and childhood periods. Despite the commonality there is still confusion about the definition and management of hypoglycaemia. Hypoglycaemia can be due to many causes (including endocrine and metabolic) in the neonatal, infancy and childhood period. For example hyperinsulinaemic hypoglycaemia is the most severe form of hypoglycaemia in the neonatal period whereas 'ketotic' hypoglycaemia presents in the childhood only during an intercurrent illness. Thus having an understanding of normal glucose physiology will not only help the clinician to understand the biochemical basis of hypoglycaemia but will also allow the clinician to organise appropriate investigations and institute the correct management. The early recognition and prompt management of hypoglycaemia is the cornerstone in preventing brain injury. During this talk I will give an overview of glucose physiology, review the causes of hypoglycaemia in the neonatal and childhood periods and discuss the different management approaches.

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CMW4.4

Autoimmune hypoglycaemia – when and how to look for anti-insulin and anti-insulin receptor antibodies

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After secretion from the pancreatic β cells, insulin exerts its pleiotropic effects by binding to its widely expressed cell surface receptor and triggering a cascade of intracellular signalling events, suppressing hepatic glucose production and inducing glucose uptake into fat and muscle among many other effects. Insulin is also cleared rapidly from the circulation, with a half-life of around 5 min, a process which is partly mediated by insulin receptor binding. This rapid clearance is critical to normal glucose homeostasis. Autoantibodies may perturb the highly dynamic glucose-insulin negative feedback loop in two major ways, both of which may lead to severe hypoglycaemia and/or hyperglycaemia. First, antibodies against the insulin receptor often have the ability to activate the receptor inappropriately irrespective of circulating insulin levels. This may

produce severe hypoglycaemia, although the chronic presence of these antibodies more commonly desensitizes the receptors, producing severe 'type B' insulin resistance. Second, high affinity, high capacity antibodies against insulin itself may perturb insulin kinetics sufficiently to produce severe hypoglycaemia associated with the presence of 'macroinsulin' complexes. Either pathological anti insulin receptor or pathological anti-insulin antibodies may arise either spontaneously or in the context of pre-existing diabetes, which may complicate interpretation of diagnostic tests. Rapid diagnosis is important, and in some cases may lead to use of potent multimodal immunosuppression to correct the severe metabolic disorder. How to select appropriate patients for antibody testing, and how best to utilise laboratory investigation to generate clinically meaningful results will be discussed.

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Applied Physiology Workshop

Digital copies: exploiting numerical models of biological systems

APW1.1

Modelling neuroendocrine systems

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In recent years, the increasing availability of massive computational capacity has reached what may be seen as a 'tipping point', bringing once unimaginable computational power into the lab. This is enabling models to be built, fit and refined during experiments, making predictive models that are powerful tools for hypothesis generation and testing. Neuroendocrine systems are at the forefront of these advances. Because of the exceptional opportunities that they offer for experimental intervention, they have long been prominent 'model systems' in neuroscience, now these model systems are the source of powerful computational models. The electrical activity of oxytocin and vasopressin cells of the hypothalamus can now be closely matched by computational models whose parameters are fit to the data by evolutionary algorithms as the data are collected. I will show how the synchronized bursting of oxytocin cells during suckling, which underlies pulsatile oxytocin secretion, can be understood through a network model of oxytocin cells, and how models can be used to explore the functional significance of the phasic firing patterns of vasopressin cells.

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APW1.2

Distinguishing normal, depressive and PTSD cortisol dynamics in humans through mathematical modelling

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PTSD is an anxiety disorder that occurs among persons exposed to a traumatic event involving life threat and injury. This is a co-morbid psychiatric disorder that occurs along with depression. Cortisol, secreted in the adrenal cortex in response to stress, is an informative biomarker that distinguishes anxiety disorders such as major depression and post-traumatic stress disorder (PTSD) from normal subjects. In comparison to normal subjects, hypocortisolemia was observed during the night in PTSD, while hypercortisolemia was observed in depressed subjects. Yehuda *et al.* proposed a hypothesis that, in humans, the hypersensitive hypothalamus–pituitary–adrenal (HPA) axis is responsible for the occurrence of differing levels of cortisol in anxiety disorders. Specifically, PTSD subjects have lower cortisol levels during the late subjective night in comparison to normal subjects, and this was assumed to occur due to strong negative feedback loops in the HPA axis. We complemented this hypothesis by constructing a mathematical model for cortisol dynamics in HPA axis using nonlinear ordinary differential equations and estimated the kinetic parameters that fitted the cortisol time series obtained from the clinical data of normal, depressed and PTSD patients. The parameters obtained from the simulated phenotypes strongly support the hypothesis that, due to disruptive negative feedback loops, cortisol levels are different in normal, PTSD and depressed subjects during the night. Bifurcation analysis carried out with the optimized parameters exhibited two supercritical Hopf points and, for the choice of parameters, the oscillations were found to be circadian in nature. Importantly, the model predicted the transitions from normal to various diseased states, and these transitions were shown to occur due to changes in the strength of the negative feedback loop and the stress intensity in the neuro-endocrine axis.

Declaration of funding

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APW1.3

Abstract unavailable.

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APW1.4

Modelling the circulating renin–angiotensin system and its effects on blood pressure

Patrick Hannaert, Vivien Aubert & François Guillaud
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In cardiovascular-renal (CVR) pathology, the renin (REN)-angiotensin (A2) system (RAS) is central as key regulator of blood pressure BP (major CVR risk factor), and it is widely targeted by therapeutical agents. CVR regulations are utterly complex: realistic and integrated models are needed. However RAS is absent or crude in existing models: in the most integrative one (Guyton's model GM), it is restricted to a simple factor acting on resistances and aldosterone: key elements are absent (e.g. REN). We integrated into GM realistic endocrine RAS modules: Plasma describing RAS actors; REN-producing JGA (juxtaglom. appar.) controlled by perfusion pressure and A2. We present such development (and late improvements).

Methods

Simulink used for modules; *M2SL* (simulation libr., Hernandez 2009) used for final, integrated GM+. *JGA*: controllers modulate REN secretion and REN-cells recruitment. *Plasma*: REN (Plasma REN Activ., PRA) & ACE kinetics, clearances are integrated to yield REN(t), A1(t) & A2(t). Parameters were optimized with Matlab tools: long-term for recruitment (5d, REN = f(Na intake)), and short-term for secretion (< 24 h, REN or ACE inhib.; Nussberger2002). Cost function = simul. − exp. root mean-squared error, normalized to data range (*nRMSE*); final validation against independant data.

Results

Stand-alone *JGA* and *Plasma* were optimized, then GM+: 9 & 23% final error for long & short-term data, resp. Simulated baseline values for PRA, REN, A1 & A2 fell within 'normal' values; so was the case when driving the model with variable sodium intake (3 mmHg per 100 mEq/d). Short term simulations of A2 infusion (1 h, 3.0 ng/kg per min) reproduced (±40%), the BP rise (6–12 mmHg) and PRA decrease (40%) (Visser2008; Gordon2000). Simulating renal artery stenosis (PP = BP-30 mmHg) showed that PRA increased strongly (four-fold) and rapidly (minutes); the subsequent BP increase brought PRA down, and was associated with a (partial, 66%) compensatory return of PP toward its initial level, allowing for a partial restoration of filtration.

Conclusion

We developed and validated a realistic endocrine RAS, featuring plasma biochemistry & regulated renin production; the resulting, integrated CVR-RAS model exhibits proper physiological and pharmacological behavior, but still requires refinement, at all levels, to capture RAS physiological complexity (e.g. tissue RAS) and approach clinical needs.

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Debate

D1

Clinical Debate: this house believes that radio iodine should be the first line treatment for all patients with Graves' disease: FOR

Jayne Franklyn
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Radioiodine is a highly effective and safe treatment for hyperthyroidism, including Graves' disease. Remission rates for Graves' hyperthyroidism following medical therapy alone, even with a full course of antithyroid drugs, are poor (~40% overall), especially in certain groups such as those with severe hyperthyroidism, those with large goitres and probably in males. Furthermore, antithyroid drugs can themselves cause significant morbidity and occasional life threatening side effects; obtaining good biochemical control can also be challenging. With increasing evidence of long term complications of hyperthyroidism, especially cardiovascular, and increasing evidence for significantly increased long term mortality from vascular diseases, prompt and effective treatment of Graves' hyperthyroidism becomes crucial. Given the excellent safety profile of radio iodine and its efficacy in curing hyperthyroidism there is a strong case for using this therapeutic option in all cases of Graves' disease, albeit with important caveats regarding timing in those with active thyroid eye disease and those desiring pregnancy or with young children in the home.

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D2

Against

Anthony Weetman
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Radioiodine is indeed the best first line treatment in all patients with Graves' disease. Except in those who want to try for pregnancy in the next few months, those with significant child care or work responsibilities that will not allow them to take the necessary radioprotection precautions, those who are breast feeding or who smoke and have ophthalmopathy, and those who have been exposed to stable iodine. Oh, and those who are not very happy to accept the risk of permanent hypothyroidism rather than have an initial trial of a treatment which will promptly and reliably reverse their hyperthyroid symptoms, while giving them an almost evens chance of a cure without hypothyroidism (a condition which we now know is not quite as appealingly straightforward to treat as it once was). In cost benefit terms there is not much in it, and in side effect terms the risks of antithyroid drug treatment need to be set against the rather large fraction of iatrogenic hypothyroidism which is not properly controlled. 'All patients' of course include children, a further Graves' subgroup in which eminence-based rather than evidence-based medicine is practised. Despite a recent call for wider use of radioiodine in childhood, the younger the child the less enthusiastic most paediatricians are to use this. In truth there is not an ideal treatment for Graves' disease and a nuanced approach which takes into full account the patient's circumstances is the only way to decide which treatment is best employed first time around.

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Meet The Expert Sessions

MTE1

Abstract unavailable.

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MTE2

Abstract unavailable.

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MTE3

Abstract unavailable.

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MTE4

Abstract unavailable.

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MTE5

Abstract unavailable.

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MTE6

Malignant phaeochromocytomas

Ashley Grossman
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The great majority of phaeochromocytomas are benign, but some 10-15% are found to be malignant, the proportion being higher when they are extra-adrenal, paragangliomas. Malignant behaviour is hard to predict, and there are few histopathological features that are consistently of use. There are novel biochemical and molecular markers, but none has proven to be as yet especially reliable although elevated urinary methoxytyramine seems to be useful. At present, size is probably as useful as any marker in predicting malignancy. I use ^{123}I-MIBG radionuclide scanning in all patients diagnosed with a phaeochromocytomas in order to identify any possible metastases pre-operatively, and as a marker of possible therapy for recurrent tumours. Where tumours are indeed malignant, it has been found that up to half show germline mutations of the *SDH-B* gene, and then family counselling and assessment is important. The standard chemotherapy for such tumours is with the combination of cyclophosphamide, vincristine and doxorubicin (CVD), but a long-term effect on survival has been difficult to demonstrate. Recent data have suggested that temozolomide may be useful a second-line chemotherapy, but responses are rarely maintained long-term. Where the tumour shows radiolabelled MIBG uptake then treatment with ^{131}I-mIBG is effective in controlling symptoms and abnormal biochemistry in many patients, and stabilization of tumour progression is often seen. Very high doses of ^{131}I-mIBG may cause tumour regression, but at the expense of considerable marrow toxicity. Our recent data also suggest an increase in myeloproliferative disorders in long-terms survivors from this treatment. Knowledge of the molecular pathogenesis of these tumours had indicated that tyrosine kinase inhibitors such as sunitinib, and mTOR inhibitors such as everolimus, may be beneficial: sunitinib may have some temporary inhibiting effect on tumour progression, but everolimus does not appear to show much therapeutic benefit. Recent *in vitro* and *in vivo* data suggest that combination therapy with broad-action mTOR and ERK inhibitors may be highly effective, but we await proper clinical trials. At present, these tumours are invariably eventually lethal, although survival can be prolonged for a considerable time by the judicial use of sequential or combination therapies.

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MTE7

Hormone misuse in sport and leisure

Richard Holt
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When humans are placed in a competitive setting, particularly in the sporting arena, they will attempt to gain an advantage over their opponent in order to win. When all legitimate methods have been exhausted and the athlete has reached their peak performance, there is a temptation for some to seek out pharmacological methods to improve performance yet further.

The earliest records of doping in sport come from ancient times but with the advent of modern pharmacology and the birth of the field of endocrinology in the 19th century, the number and quantity of drugs used to improve strength and overcome fatigue increased dramatically.

Doping not only damages the integrity of sport but may cause significant harm to athletes who use performance enhancing drugs.

This workshop will describe most commonly abused performance enhancing drugs, including insulin. The potential beneficial and adverse effects will be discussed. The workshop will finally discuss the therapeutic use exemption (TUE), which is required for all elite competitors who use banned substances for clinical reasons.

Declaration of funding
The GH-2004 project has received funding from the World Anti-Doping Agency, US Anti-Doping Agency and the Partnership for Clean Competition.

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MTE8

Nanometre-resolution imaging of hormonal secretion in living cells in real time

Rory Duncan
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Biochemists have defined a probably complete catalogue of proteins involved in insulin secretion. Similarly, over the last two decades, biochemists and physiologists have defined the physical characteristics of different types of ion

channels that underlie normal pancreatic beta cell physiology. What is missing is information describing the 'wheres and whens': where are these proteins (i.e. not just for example on the surface of a cell, but how are the single protein molecules arranged) and when do they act? Recently, it has become possible to examine the nano-scale locations, movements and interactions of 1000 s of single molecules inside living cells. We will provide high quality training to the student, to define with the highest possible resolution where the relevant ion channel molecules are in cells, when they interact with other proteins, how they move around, when they are active and address the important question; where and when do these molecules contribute to secretion in neuroendocrine cells?

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MTE9

Abstract unavailable.

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Nurse Session

The Patient Pathway for Pituitary Care

N1.1

Abstract unavailable.

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N1.2

Abstract unavailable.

DOI: 10.1530/endoabs.31.N1.2

N1.3

Abstract unavailable.

DOI: 10.1530/endoabs.31.N1.3

N1.4

Abstract unavailable.

DOI: 10.1530/endoabs.31.N1.4

N1.5

Abstract unavailable.

DOI: 10.1530/endoabs.31.N1.5

Late Effects

N2.1

Abstract unavailable.

DOI: 10.1530/endoabs.31.N2.1

N2.2

Abstract unavailable.

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N2.3

Gonadal late effects

Maralyn Druce
Barts and the London Medical School, London, UK.

Over recent decades, survival rates have improved for cancers in both children and adults. In 2009 it was estimated that approximately two million people were living with or beyond cancer in the UK, rising by 3% a year. Patients are generally aware of the short-term side effects of cancer therapies but 'late effects' of cancer treatment, occurring months to years after therapy is completed, are less well appreciated. Detailed and large-scale longitudinal studies have shown that the endocrine system is a frequent casualty of cancer therapies and an understanding of these outcomes may help us to optimise treatments. This may help us to maintain benefits while reducing long-term risks. This knowledge also helps us to support our patients in preparation and planning for their future.

We will consider several areas for discussion including:

- The effects of cancer and its treatment in childhood on the later gonadal function and reproductive capacity of male and female adult survivors.
- How the treatment of various cancers in adulthood affects gonadal function and reproductive capacity of both male and female cancer survivors.
- Who is at particular risk, whether the risk can be predicted and whether there are strategies for damage limitation and fertility preservation in both men and women who are being treated for cancer.
- The management of hormone replacement therapy in adult men and women who have hypothalamo-pituitary-gonadal axis dysfunction after treatment for cancer. Are there particular risks of such therapy in these individuals?
- How we can counsel and help men and women to make decisions about their management based on the evidence that we have available.

We will also consider whether published guidance is available to help us and what further information might be useful to gather in the future.

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Young Endocrinologists' Session

Young endocrinologists' prize lectures

YEP1.1

Clinical and pre-clinical studies of neuroendocrine tumours (NETs) in multiple endocrine neoplasia type 1 (MEN1), and evaluation of MEN1 gene replacement therapy for MEN1-associated NETs.

Gerard Walls[1,2], Paul Newey[1], Manuel Lemos[1], Mahsa Javid[1], Sian Piret[1], Anita Reed[1] & Rajesh Thakker[a]
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We have studied clinical and pre-clinical models to investigate neuroendocrine tumour (NET) development and efficacy of novel therapy for NETs. We focused on multiple endocrine neoplasia type 1 (MEN1), an autosomal dominantly inherited condition characterised by the combined occurrence of pancreatic islet and anterior pituitary NETs with parathyroid and adrenocortical tumours. MEN1 is due to MEN1 gene mutations that inactivate Menin, a tumour suppressor. Our clinical studies revealed the unexpected occurrence of >2 cm non-functioning pancreatic NETs in $>15\%$ of asymptomatic children with MEN1, leading to updated international guidelines for pancreatic NET screening and detection by 10 years of age in MEN1 patients. Treatment of MEN1-associated foregut carcinoids, pancreatic and pituitary NETs is more difficult than for equivalent tumours in non-MEN1 patients as they are larger, multiple, more aggressive, have a higher prevalence of metastases, and resist chemotherapy or radiotherapy due to low proliferation rates. Therefore, to facilitate development of better and alternative therapies for MEN1-associated NETs, we established a knockout mouse model for MEN1 and novel *in vivo* methodologies to: evaluate NET proliferation using long-term five-bromo-two-deoxyuridine administration in drinking water; mathematically model NET growth kinetics; and MRI for NET imaging. Our pre-clinical studies revealed that homozygous loss of Men1was embryologically lethal and influenced by genetic modifiers. However, heterozygous (Men1+/−) mice developed pancreatic, pituitary, parathyroid, and adrenocortical tumours with hypercalcaemia, hypophosphataemia and hypercorticosteronaemia. Furthermore, NETs had loss of heterozygosity for Men1 and loss of menin expression, whilst proliferation rates were $<2\%$ and followed second-order kinetics. Therefore, we undertook a blinded, randomised-controlled trial of Men1 gene therapy for treating Men1+/− pituitary NETs using a recombinant replication-deficient adenoviral vector expressing wild-type Men1. Treated NETs demonstrated in vivo expression of menin and inhibition of proliferation, without significant adverse effects or increased mortality, and established pre-clinical proof-of-concept for gene replacement therapy in pituitary NETs.

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YEP1.2

The Wnt/β-catenin effector Tcf3/TCF7L1 is required for normal hypothalamic–pituitary development

Carles Gaston-Massuet[1,2], Mark McCabe[2], Chun-I Wu[3], S. Neda Mousavy Gharavy[1], Markella Koniordou[1], Bradley J Merrill[3], Mehul Dattani[2] & Juan Pedro Martinez-Barbera[1]
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The pituitary gland, is a small midline organ situated at the base of the brain, that acts as a master regulator of multiple physiological functions: such as growth, puberty, metabolism, stress response, reproduction and lactation. The pituitary gland is composed of three lobes: the anterior and intermediate, which form the anterior pituitary (AP and contains hormone producing cells), and the posterior lobe which constitutes the posterior pituitary (PP contains axonal inputs). Many molecules that govern the development of the AP have been identified, and mutations within a number of these molecules have been shown to cause varying pituitary phenotypes, from congenital hypopituitarism to pituitary tumours. Congenital hypopituitarism encompasses a range of disorders that can be manifested as an isolated hormone deficiency, or loss of multiple hormones (combined hypopituitarism), in which two or more hormones are lacking. Severe endocrine dysfunction can also result from pituitary tumours, such as adamantinomatous craniopharyngiomas (ACPs). ACPs are slow-growing tumours that arise from the RP, affect mainly children and often cause lesions in the nearby structures – the hypothalamus and the optic nerves – with life-threatening consequences and high morbidity for the patients. Previously, we have shown that the Wnt/β-catenin pathway needs to be antagonised during early AP development to maintain the appropriate numbers of progenitor cells. Hence, absence of two Wnt/β-catenin antagonising genes, *Six3* and *Hesx1*, in the AP results in a higher proliferation of undifferentiated precursors, severe AP hyperplasia and dwarfism (Gaston-Massuet, Dev Biol, 2008). With this in mind, we studied the effect of the absence of the Wnt/β-catenin downstream effector *Tcf3* by conditionally ablating Tcf3 from the AP ($Hesx1^{Cre/+}$; $Tcf3^{flox/flox}$). We show for the first time a novel role for *Tcf3* in the AP development. Absence of Tcf3 results in AP hyperplasia a phenotype that closely resembles that of *Hesx1* mutants. Moreover, morphological analyses of second murine model that carries a Tcf3 allele that lacks β-catenin binding domain, $Tcf3^{\Delta N/\Delta N}$, shows normal pituitary development indicating that the function Tcf3, is to repress downstream targets. In contrast, over-activation of the canonical Wnt pathway, by conditional expression of a degradation-resistant form of β-catenin ($Ctnnb1^{flox(ex3)}$) in the undifferentiated precursors of the pituitary gland ($Hesx1^{Cre/+}$; $Ctnnb1^{flox(ex3)/+}$) results in hypopituitarism, severe hyperplasia and adamantinomatous craniopharyngioma ACP- like tumours. This finding, demonstrated for the first time, a causal effect of mutations in β-catenin in ACP, and provided with and novel animal model to further study the ACP pathogenesis (Gaston-Massuet PNAS, 2011). Importantly, only pituitary undifferentiated precursors/stem cells are responsive to mutated β-catenin, which identifies the cell origin of ACP tumours to be pituitary undifferentiated precursor cells that are $Sox2^{+ve}$, $Sox9^{-ve}$, $p27Kip2^{+ve}$. In order to identify a possible therapeutic effects of Wnt/β-catenin inhibition *in vivo*, we have generated a mouse that antagonises Wnt by expressing *Hesx1* from the Rosa26 locus ($Hesx1^{Cre/+}$; $Ctnnb1^{flox(ex3)/+}$; $R26^{Hesx1/+}$). Interestingly, this triple compound mice show a median survival of 29 weeks compared to 12 weeks for the double compound mutants ($Hesx1^{Cre/+}$; $Ctnnb1^{flox(ex3)/+}$) indicating that *in vivo* inhibition of Wnt results in amelioration of adamantinomatous craniopharyngioma tumours. Over-expression of *Hesx1* in these tumours leads to restoration of wild-type levels of *Lef1* and *Axin2* (downstream targets of β-catenin) and proliferation, indicating that this inhibition is sufficient to exert and ameliorating effect on ACPs. We have generated an *in vitro* murine ACP-cell culture assay to identify Wnt β-catenin inhibitory compounds with potential therapeutic effect on ACPs. We have found, that indomethacin and sulindac sulfone have a strong effect on cell colony assay growth, suggesting a positive therapeutic effect of these compounds. Our research indicates that Wnt/β-catenin pathway is crucial for pituitary development. Initially, this pathway needs to be negatively regulated to maintain the progenitor pool. When Wnt/β-catenin is aberrantly expressed by activating mutations, this leads to pituitary tumours such as ACPs.

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Maintaining your endocrine career despite what life throws at you – Keeping everything up in the air

YE1.1

Maintaining an active basic research career whilst juggling an academic and personal life

Gareth Lavery
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We all want to be earnest and successful basic researchers; usually this means running a group and this can be difficult when as an academic you will also have administrative duties, committees to attend and teaching to give. And as a rounded and fulfilled human being you will have a personal life that rewards in many other ways, such as a passion for sport, family or other activities. Life is and should be fluid, so it is unrealistic and unrewarding to schedule an equal number of hours for each of your various work and personal activities, you have to use common sense, get support from friends and family, involve mentors and managers and offer it in return, to ultimately balance all the important things in life.

For some, working long hours creates value and balance in their lives. For others, it is not a routine they can productively or enjoyable maintain. A good work-life balance for someone who has no children may be different than that of someone with children. The best work-life balance for you may be different than that of your co-workers or your manager.

We will explore some ways in which we can balance our academic and personal lives so that we are fulfilled at all levels and therefore better equipped to continue being contented and successful scientists. We will also discuss how building

around you a team of trusted and dependable colleagues, supportive family, friends, mentors and managers will provide balance, harmony and productivity. It is important that you, as an individual, find ways to create the right work-life balance for yourself.

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YE1.2

Juggling a clinical workload: and academic research

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A number of challenges are faced by those wishing to pursue a career as an academic clinician. Perhaps the most obvious hurdle for those at a junior stage of research career, is achieving sufficient academic success to attract ongoing research funding, whilst achieving the clinical standards required for the completion of training and in due course, revalidation. The introduction of the integrated academic clinical training program has helped address this balance, improving opportunities for research at junior stages of clinical training, and raising the profile of academic careers within the modern NHS. The potential rewards of an academic career are significant including ongoing intellectual stimulation, freedom to pursue ones own ideas and interests, travel and opportunities for collaboration. However, the journey may not be straightforward and enthusiasm, persistence and willpower are required to deflect worries over job insecurity, time-pressures, and a feeling of being 'behind' ones peer-group. Furthermore, a clear focus on ones goals is required, including frequent reassessment of the 'where am I going?' and 'what do I need to do to get there?' questions. Other skills may be developed during this period including working efficiently, prioritizing and planning, negotiating and to some extent, managing uncertainty. Identifying independent mentors may be hugely beneficial and may help overcome many of the challenges faced. However, the ideal outcome is a synergistic balance between clinical and academic work; using patients to inspire important research questions, and applying research methodology and an inquisitive mind to the provision of clinical care.

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YE1.3

Abstract unavailable.

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YE1.4

Non-traditional career paths towards an academic career

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Many post-doctoral scientists still believe that the road to academic success follows the traditional university career trajectory. Although some lectureship and fellowship positions will inevitably be filled by those who have chosen this path, forward-thinking universities now seek principal investigators who have international collaborations and industry links, supported by unique expertise and knowledge on many divergent aspects of scientific research. But as the economic downturn bites, leading to a drop in research funding, how does the aspirational scientist differentiate their academic credentials in this highly-competitive career route?

One option sometimes misunderstood by post-doctoral researchers is the academic-related openings found within pharmaceutical industries. As 'Big Pharma' continues its withdrawal from the early stages of research and development, there has been a noticeable increase in the growth of contract research organisations (CROs) and academic spin-out companies. Taking these employment avenues can provide ample opportunities for driven scientific researchers to gain significant experience working at the academic-industry-commercial interface. This knowledge is of growing interest to universities. They increasingly expect the 'bench-to-bedside' approach to research projects, something traditionally associated with pharmaceutical companies, as this lends a strong translational aspect to the work, and this, in turn, attracts grant funding. However, there are many pitfalls to avoid with such a career path. For example, how do budding academics continuously publish high-quality research articles that may compromise the commercial interests of the company? Without a previous track record, how do they demonstrate an ability to attract research funding?

This session will answer these questions and provide insights into how a career in industry, rather than being the death of your academic career, can be the springboard into those difficult to get lectureship or fellowship posts.

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YE1.5

Daphne Jackson Fellowships offer returners the chance to re-establish a research profile after a career break

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This presentation will describe the barriers, the business case and the benefits of employing returners to science, engineering and technology (SET) careers by the only organisation in the UK solely dedicated to returning scientists to careers. The Daphne Jackson Trust is an organisation that offers Fellowships to men and women who have taken a career break from science, engineering or technology. With falling numbers of graduates entering SET careers, employers in both academia and industry can no longer afford to ignore the fact that scientists who take a career break often do not return to their old jobs. This has serious implications for the cost of recruitment and training of staff and, overall, is affecting the competitiveness and productivity of many Universities and companies in the UK and Europe. Scientists often feel unable to return due to a wide variety of reasons: lack of part-time or flexible posts, difficulties with childcare, unpleasant and outdated working environments, lack of career progression, secretive and unfair recruitment and promotion procedures, to name but a few.

In fact, it can be almost impossible for many to return without the help of a Daphne Jackson Fellowship, as it offers the opportunity to re-establish scientific credentials and obtain a recent research record whilst retraining and renewing skills that are essential for a future career. The Trust has a 96% success rate in returning Fellows to SET based careers and these returners have much to offer their employers. Not only are they fully qualified for the role in the first place but their career breaks have often heightened the skills required by top class employers: time management, flexibility and adaptability, conflict resolution and working under pressure. It is time that all Universities and Companies became engaged in schemes such as that offered by the Daphne Jackson Trust.

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YE1.6

Abstract unavailable.

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Senior Endocrinologists Session

SE1.1

Abstract unavailable.

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SE1.2

Robert Graves' and his remarkable colleagues

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The 19th century saw the emergence of an extraordinarily gifted group of Dublin doctors who enjoy eponymous recognition. Immediately on graduating Robert Graves' travelled for over 2 years to many of the foremost medical centres in Europe. On his return he introduced formal bedside teaching and daily lectures which has become the model for clinical training in these islands. Graves' main other interest was the treatment of febrile illness recognizing the importance of nutrition, hydration and contagion. Graves' was a prolific author but his only contribution to the disorder which bears his name was a single paper and this was not the first description of hyperthyroidism. William Wilde and William Stokes were colleagues, friends and the earliest biographers of Graves'. Wilde was the most eminent ear specialist of his day, author, editor, antiquarian, medical advisor for first Irish censuses and father of Oscar. Wilde was nominated by Graves' to write his 'portrait' but confined him to medical matters. Ten years after Graves' death Stokes wrote a biography which described the young Graves' as dashing, decisive, a charismatic leader and a teller of tales. Stokes is remembered eponymously in association with contemporaries Adams and Cheyne. Towards the end of Graves' career he was involved in a fractious debate with Dominic Corrigan concerning remuneration of dispensary doctors who treated famine victims (which Graves' considered to be inadequate) and also the management of coincident typhus. Corrigan was recognized internationally for his description of aortic insufficiency. However his nomination for Honorary Fellowship of the Royal College of Physicians of Ireland was blackballed, probably by Graves'. Graves' retired from his posts as professor and physician before he was 50 years. As Graves' withdrew Corrigan rose to prominence. After Graves' death at 53 years, Corrigan became one of the College's most influential presidents.

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SE1.3

Should the aging male become a father?

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Couples in developed countries are increasingly delaying child bearing to later in life. While it is well known that female reproductive functions decrease and genetic risks for the offspring increase beyond the age of 35 and seize completely around the age of 50, the influence of risks of paternal age on fertility and offspring are less well known. Indeed, until recently life long fertility was assumed for the male. However, testicular function slowly declines with age and fertility decreases significantly after age 40. Moreover, advancing paternal age is associated with increased rates of early abortion. Children of older fathers also have a higher risk of autosomal dominant genetic disorders such as archondroplasia, Apert syndrome and neurofibromatosis, as well as complex diseases such as schizophrenia, depressions and autism spectrum disorders (ASD). Although these risks remain small couples should not only be informed about maternal age-related changes in reproductive functions, but also about the paternal age-related decline in fertility and paternally-mediated risks for the offspring. These facts should also serve to encourage couples to procreate at a younger age.

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SE1.4

Hypovitaminosis-D and the RAS in type 2 diabetes risk

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Background
Hypovitaminosis D is associated with T2DM risk, cross-sectionally and prospectively; supplementation can reduce insulin resistance and increase glucose-induced insulin secretion in humans. Increased pancreatic islet RAS activity is induced by hyperglycemia, enhancing β cell damage (Leung PS *et al.*). Vitamin D is now known to suppress renin secretion, thus we have examined effects of calcitriol on increased islet RAS activity *in vitro* ± hyperglycemia and the effects of RAS blockade on islet function during continuing vitamin D deficiency *in vivo*.
Methods
i) RAS component expression and secretion in islets from WT mice were examined under normal and hyperglycemic conditions and in VDR-KO mice. ii) RAS activity and β cell function were examined in islets from mice with dietary vitamin D deficiency ± treatment with aliskrenin.
Results
i) Adding calcitriol to islets before or during exposure to hyperglycemia prevented increases in RAS activity and related disorders, and restored insulin secretion (effects that were optimal @10^{-9} molar). ii) RAS blockade *in vivo* during vitamin D deficiency restored glucose tolerance, reduced overall and postprandial insulin resistance whilst also reducing islet RAS over-activity and related disorders
Comments
Upregulation of RAS activity contributes to islet dysfunction, probably explaining T2DM risk-reduction in RCTs of RAs blockers. Hypovitaminosis D contributes to T2DM risk through avoidable effects on islet RAS activity. Vitamin D and RAS blockade may be additive or synergistic for T2DM risk reduction.
Preliminary studies suggest vitamin D suppression of hepatic RAS may reduce insulin resistance.
RAS suppression may benefit RAS-increasing diabetic complications, like proliferative retinopathy.
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SE1.5

Type 1 and type 2 diabetes are the same disorder of insulin resistance, but with different genetic backgrounds

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Some 40 years ago, diabetes was re-classified from a single disorder into autoimmune (T1D) and metabolic (T2D) on the interpretation of observation rather than the outcome of experiment. The 20 or so experiments carried out since to test the autoimmune hypothesis (largely randomised trials of immunotherapy) have proved disappointing, and none has translated into patient benefit. There is arguably reason to question the autoimmunity paradigm. The accelerator hypothesis argues that T1D and T2D are the same disorder of insulin resistance, set against different genetic backgrounds. Humans are born with a substantial reserve of β cells, which is gradually lost throughout life. For most, the rate of loss is slow and inconsequential. When accelerated, however, the loss becomes critical within a lifetime, and diabetes results at an age (adulthood or childhood) determined by the tempo of loss. The accelerator hypothesis makes no fundamental distinction between 'no diabetes' (the majority), 'slow diabetes' (adult onset) and 'fast diabetes' (childhood onset). What matters to prevention is proper identification of the accelerator responsible. Insulin demand (insulin resistance) is the primary accelerator, and autoimmunity the response to stressed beta cells in the few with reactive HLA genes which further accelerates their loss. Evidence will be presented that pre-type one diabetic children are insulin resistant, that autoantibodies (the hallmark of T1D) are common in T2D and that the age at presentation of T1D in children is inversely related to their BMI – true acceleration. A series of trials to test the accelerator hypothesis are planned.

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SE1.6

Suspicious hypoglycaemia; was it insulin?

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Hypoglycaemia – especially in elderly hospital in-patients – is far less uncommon in non-diabetic patients than was previously thought but is only very rarely due to accidental or malicious 'insulin' (including insulin analogues and sulphonylurea) administration. The question of when to suspect that this might be the case and how to confirm or refute it is one that crops up from time to time in every community. Sometimes it is relatively simple and easy to confirm as, for example, when the patient is alive when first seen, relevant blood samples have been collected and the appropriate analyses ie for plasma glucose, insulin, C-peptide, proinsulin, β-hydroxybutyrate, insulin-antibodies and sulphonylurea concentrations, have been carried out on them. More often it is extremely difficult or impossible, especially in retrospect or if the patient is dead when first seen and reliance is placed on retrospective analysis of the clinical case notes. Equally difficult is identifying the culprit in cases of established misfeasance whether due to accident or negligence or to malicious (including misguided mercy killing) insulin administration. In such cases conviction or exoneration depends heavily upon the quality of advocacy and the persuasiveness of expert opinion in court.

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Oral Communications

Young Endocrinologists prize session

OC1.1

TNFα directly regulates *in vivo* corticosteroid metabolism in inflammatory arthritis

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Within the synovium of patients with rheumatoid arthritis (RA), synovial fibroblasts generate active corticosteroids through expression of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). *In vitro*, this enzyme is strongly up-regulated by pro-inflammatory cytokines such as tumour necrosis factor α (TNFα) and IL1β. In this study, we determined the relationship between inflammation and global 11β-HSD1 activity *in vivo*, in a clinical study of patients with inflammatory arthritis treated with anti-TNFα therapy. Urine samples were collected from RA ($n=20$) and psoriatic arthritis (PsA) ($n=20$) patients as part of a multicentre study assessing responses to infliximab and etanercept and from healthy controls (HC, $n=51$). Systemic measures of glucocorticoid metabolism were assessed by gas chromatography/mass spectrometry at week 0, 4 and 12 of anti-TNFα therapy and calculated as the tetrahydrocortisol + allotetrahydrocortisol/tetrahydrocortisone ((THF + alloTHF)/THE) and the cortols/cortolones ratios. Clinical data including DAS28 and CRP were also collected. Urinary (THF + alloTHF)/THE (1) and cortols/cortolones (2) ratios were significantly higher in RA and PsA patients prior to treatment compared to HC (1: RA, 1.22 (0.93–1.3), $P=0.005$; PsA, 1.05 (0.87–1.41), $P=0.02$; HC, 0.91 (0.75–1.05); 2: RA, 0.66 (0.51–0.75), $P=0.0001$; PsA, 0.60 (0.51–0.66), $P=0.0005$; HC, 0.48 (0.41–0.54)). The elevated (THF + alloTHF)/THE ratio fell following anti-TNFα therapy at 4 weeks for RA (1.22 (0.93–1.30) vs 0.94 (0.81–1.23), $P=0.017$) and at 12 weeks for PsA (1.05 (0.87–1.41) vs 0.96 (0.72–1.23), $P=0.018$). A similar observation was made for the cortols/cortolones ratio at 4 and 12 weeks for RA (0.66 (0.51–0.75) vs 0.55 (0.51–0.62), $P=0.004$ and 0.66 (0.51–0.75) vs 0.56 (0.50–0.62), $P=0.03$). In patients with RA there was a positive correlation between the 12 week change in DAS28 score (1), CRP (2) and the 12 week change in the cortols/cortolones ratio (1: $r=0.64$, $P=0.003$; 2: $r=0.45$, $P=0.048$). This study demonstrates for the first time that TNFα plays a central role in regulating 11β-HSD1 activity *in vivo* in patients with inflammatory arthritis.

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OC1.2

Macrophage-specific 11β-hydroxysteroid dehydrogenase type 1 deficiency promotes angiogenesis but impairs resolution of K/BxN serum induced arthritis

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Chronic inflammatory disease is often accompanied by angiogenesis and fibrosis. Glucocorticoids (GCs) exert anti-inflammatory and anti-angiogenic effects, in which macrophages are a major target. Local endogenous GC action is controlled by 11β-hydroxysteroid dehydrogenase (11β-HSD), with the type 1 isozyme, 11β-HSD1 converting inactive GCs into active forms. Mice deficient in 11β-HSD1 have a phenotype consistent with reduced glucocorticoid action, including increased angiogenesis and more severe acute inflammation.

To elucidate the role of 11β-HSD1 in macrophages, MKO mice with conditional disruption of 11β-HSD1 in macrophages were generated by crossing *LysM-Cre* with *Hsd11b1*$^{flox/flox}$ mice. Cre-negative littermates were controls. 11β-HSD1 reductase activity was reduced by 82% in resident peritoneal macrophages of MKO mice.

To investigate angiogenesis, sponge implants were inserted subcutaneously into each flank of adult male mice and harvested after 21 d. Chalkley counting on H&E stained sponge sections showed significantly increased angiogenesis in MKO mice (score: 5.2 ± 1.0 vs 4.3 ± 0.7; $P<0.05$, $n=9$–11). *Cdh5* expression (encoding VE-cadherin) was higher in sponges from MKO mice (relative expression: 1.5 ± 0.9 vs 0.8 ± 0.6; $-P<0.05$), as was *Il1b* (relative expression: 6.5 ± 6.4 vs 1.5 ± 0.9; $P<0.05$). *Vegfa* mRNA was unchanged.

Inflammation was investigated following i.p. injection of 125 µl K/BxN serum to induce arthritis. Onset of inflammation (d1-8) was similar to controls ($n=6$–7). MKO mice exhibited greater clinical inflammation scores in the resolution phase of arthritis (d13–21; area under the curve: 86.6 ± 14.7 vs 60.1 ± 13.4; $P<0.005$), indistinguishable from global 11β-HSD1-deficient mice. H&E staining revealed pronounced fibroplasia predominantly in the supporting mesenchyme associated with the tenosynovium.

These data suggest that intracellular regeneration of active glucocorticoids by 11β-HSD1 in macrophages is crucial to promote resolution of inflammation and limit fibro-proliferation. Moreover, macrophage 11β-HSD1 contributes to the anti-angiogenic effects of endogenous glucocorticoids. Targeted delivery of inactive glucocorticoid precursors to macrophages may be of benefit in chronic joint inflammatory disease.

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OC1.3

11β-HSD1KO mice are protected from glucocorticoid dependent age-associated muscle atrophy

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Glucocorticoids (GCs) are prescribed for their anti-inflammatory and immunosuppressive properties. However, they have significant side-effects including a decline in muscle mass and function which has similarities to age related sacropaenia. Within skeletal muscle 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts 11-dehydrocorticosterone (11DHC) to active corticosterone (CORT) amplifying local GC action. We hypothesise that 11β-HSD1 mediated intramyocellular GC generation may contribute to sarcopenia. To investigate this we assessed 6-week-old male wild-type (WT) mice treated with CORT (100 µg/ml), 11DHC (100 µg/ml) or vehicle via the drinking water for 5 weeks, and young (26 weeks) and aged (112 weeks) WT and 11β-HSD1KO mice and assessed grip strength as a marker of muscle function and assessed muscle gene expression profiles using fluidigm expression arrays.

In WT mice, both CORT and 11DHC increased the expression of the key muscle atrophy genes including the FOXO1&3 transcription factors, MuRF1, atrogin-1, myostatin, GSK3β and GADD45a in quadriceps muscles. This was paralleled by decreased quadriceps weight and grip strength compared to vehicle treated and young mice. WT mice at 112 weeks of age also demonstrated increased expression of the same atrophy gene expression profile in quadriceps muscles as seen in CORT treated mice, paralleled by an age-dependent decrease in grip strength. However, aged 11β-HSD1KO mice were protected from the atrophy associated gene expression profile of increased FOXO1&3, MuRF1, atrogin-1, myostatin, and GSK3β expression, and crucially these mice retained a muscle mass and grip strength reminiscent of a younger mouse.

In summary, we have identified a muscle gene expression profile common to both GC and age associated myopathy, which 11β-HSD1KO mice do not display associated with increased muscle mass and function. These data suggest that muscle expression of 11β-HSD1 could offer a novel therapeutic target preventing GC-related myopathy and sarcopaenia, ultimately improving healthy lifespan.

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OC1.4

A serum microRNA profile potentially associated with glucocorticoid mediated insulin resistance

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Patients with glucocorticoid (GC) excess develop insulin resistance and central obesity. We have demonstrated that GCs have tissue-specific effects on insulin sensitivity in humans, causing resistance in skeletal muscle but sensitivity in subcutaneous adipose tissue. The molecular mechanisms that underpin these differences remain poorly understood. Over the last decade small non-coding RNAs (microRNAs–miRNAs) controlling protein expression have been identified, representing an additional regulatory layer to the control of metabolism through the regulated expression of enzymes, transcription factors and signalling components. miRNAs are readily detected in human serum and altered miRNA profiles have been linked to metabolic disease.
In order to identify GC regulated miRNAs blood was extracted from 10 healthy volunteers under four treatment conditions. Volunteers were fasted for 12 h and infused with either saline or hydrocortisone (0.2 mg/kg per h) this was followed by 4 h of insulin infusion (100 mU/m^2 per min). Samples were taken after fasting (+/− hydrocortisone) and after insulin infusion (+/− hydrocortisone). RNA was extracted and used in miRNA array analysis, providing full coverage of mirBASE17, including 1750 known human miRNAs.
In the fasting state, hydrocortisone treatment significantly altered serum levels of seven miRNAs, including some with predicted metabolic targets. Compared to fasting saline, the combination of hydrocortisone and insulin regulated 16 miRNAs, interestingly increasing miR-195 (associated with hypertension) and miR-144 (inhibition of insulin receptor substrate 1 (IRS1)). Compared to insulin alone, hydrocortisone regulated 25 miRNAs, interestingly increasing miR-637 (involved in adipocyte differentiation) and miR-145 (inhibition of IRS1 and 2). This study has identified novel profiles of GC regulated miRNAs in human serum associated with insulin sensitivity, a number of which have predicted and demonstrated metabolic targets. These data will allow us to investigate the endocrine regulation of miRNAs and their role in metabolic homeostasis and highlights potential miRNA targets that may underpin the tissue-specific effects of GCs on insulin action.

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OC1.5

Inhibition of 5α-reductase type 1 with dutasteride impairs insulin sensitivity

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5α-Reductase (5αR) inhibitors decrease prostatic dihydrotestosterone in benign prostatic hyperplasia (BPH) treatment; finasteride inhibits 5αR type 2, while dutasteride inhibits 5αR1 and 2. 5αRs, especially 5αR1, are also expressed in metabolic tissues regulating actions of androgens and other substrates, including glucocorticoids.
Hypothesis
5αR1 inhibition with dutasteride induces metabolic dyshomeostasis.
Study
With ethical approval, in a double-blind RCT, we studied metabolism in 47 men (20–85 years) before and after 3 months of either dutasteride (0.5 mg daily; $n=$ 17; D), finasteride (5 mg daily; $n=$ 16; F) or control (tamsulosin; 0.4 mg daily; $n=$ 14). The primary outcome was insulin sensitivity, measured during a two-step (10; 40 mU/m^2 per min) hyperinsulinaemic euglycaemic clamp, with d2-glucose and d5-glycerol tracers. Data are mean (95% CI; *P* value) difference in change from baseline, compared by one-way ANOVA with LSD *post-hoc* tests where appropriate.
Results
D, but not F, impaired insulin sensitivity. During high-dose insulin, the *M* value (mean steady state glucose infusion rate) decreased with D vs both control and F, by 10.1 mg/kg fat-free mass/min (−16.3; −3.9; $P=0.002$); signifying impaired skeletal muscle insulin sensitivity. Glucose and glycerol rates of appearance during low-dose insulin were unchanged. Tracer infusion alone induced hyperinsulinaemia only with D by 11 pmol/l (3; 20; $P=0.009$). D increased HOMA-IR and fasting C-peptide by 15% (3; 27; $P=0.015$), and 114.5 pmol/l (31.5; 197.6; $P=0.007$) respectively. Fasting glucose, cholesterol, body mass index, waist:hip ratio and blood pressure were unaltered. Body fat (bioimpedance) increased 2.6% (0.9; 4.2; $P=0.003$) with D. Post-treatment visceral and subcutaneous adipose volumes (magnetic resonance imaging; L4/5), and hepatic fat (^{1}H spectroscopy), were unchanged. Serum adiponectin, resistin, IL8, and MCP1 were unchanged, however leptin increased 44% (16; 73; $P=0.03$) with D. In all indices F was not different to control.
Conclusion
5αR inhibition with dutasteride, but not finasteride, impairs peripheral (principally muscle) insulin sensitivity, and increases body fat and leptin. 5αR1 inhibition is potentially detrimental to metabolic health; this may have important implications for BPH treatment.
Declaration of funding
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OC1.6

Improving the vitamin D status of vitamin D deficient adults is associated with improved mitochondrial oxidative function in skeletal muscle

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Objective
Suboptimal mitochondrial function has been implicated in several disorders where fatigue is a prominent feature. Vitamin D deficiency is a well-recognised cause of fatigue and myopathy. The aim of this study was to examine the effects of cholecalciferol therapy on skeletal mitochondrial oxidative function in symptomatic, vitamin D deficient individuals.
Design
This longitudinal study assessed mitochondrial oxidative phosphorylation in the gastro-soleus compartment using phosphorus-31 magnetic resonance spectroscopy measurements of phosphocreatine recovery kinetics in 12 symptomatic, severely vitamin D deficient subjects before and after treatment with cholecalciferol (10–12 weeks later). All subjects had serum assays before and after cholecalciferol therapy to document serum 25OHD and bone profiles. 15 healthy controls also underwent ^{31}P-MRS and serum 25OHD assessment.
Results
The phosphocreatine recovery half-time ($\tau_{1/2}$PCr, $\tau_{1/2}$ADP) was significantly reduced following cholecalciferol therapy in the subjects indicating an improvement in maximal oxidative phosphorylation ($P<0.001$, $P=0.003$). This was associated with an improvement in mean serum 25OHD levels (8.8±4.2 to 113.8±51.5 nmol/l, $P<0.001$). There was no difference in phosphate metabolites at rest. A linear regression model showed that decreasing serum 25OHD levels are associated with increasing $\tau_{1/2}$PCr ($r=-0.41$, $P=0.009$). All patients reported an improvement in fatigue following cholecalciferol therapy.
Conclusions
Cholecalciferol therapy augments muscle mitochondrial maximal oxidative phosphorylation following exercise in symptomatic, vitamin D deficient individuals. This finding suggests that changes in mitochondrial oxidative phosphorylation in skeletal muscle could at least be partly responsible for the fatigue experienced by these patients. For the first time, we demonstrate a link between vitamin D and the mitochondria in human skeletal muscle.
Declaration of funding
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OC1.7

Autosomal dominant hypocalcemia type 2 is caused by germline GNA11 gain-of-function mutations

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The calcium-sensing receptor (CaSR) is a guanine-nucleotide-binding protein (G-protein)-coupled receptor that has a central role in calcium homeostasis. Loss-of-function mutations of the CaSR result in familial hypocalciuric hypercalcemia

type 1 (FHH1) and gain-of-function mutations in autosomal dominant hypocalcemia (ADH). Recently, loss-of-function $G\alpha_{11}$ mutations have been identified to cause FHH2 and we hypothesised that gain-of-function $G\alpha_{11}$ mutations may be a cause of ADH in the ≈60% of ADH patients who do not have CaSR mutations. DNA sequence analysis of the 1077 bp coding region and 12 exon-intron boundaries of *GNA11* in eight patients with hypocalcaemia but without *CaSR* mutations identified two missense mutations, Arg181Gln and Phe341Leu. These missense mutations were absent from ≈5400 exomes and both Arg181 and Phe341 residues are highly conserved in vertebrate $G\alpha_{11}$ subunit orthologues and human paralogues indicating that these were likely mutations of *GNA11*. Wild-type (WT) and ADH2-associated mutants Gln181 and Leu341 $G\alpha_{11}$ proteins were expressed by transient transfection in HEK293 cells, stably transfected with CaSR, and assessed by measuring their intracellular calcium responses to changes in extracellular calcium ($(Ca^{2+})_o$). This revealed that expression of the mutant $G\alpha_{11}$ proteins resulted in a leftward shift in the concentration-response curves to alterations in $(Ca^{2+})_o$ and a significant ($P<0.0001$) reduction in EC_{50} when compared to transfection with WT $G\alpha_{11}$ (WT $EC_{50}=2.25$ mM (95% confidence interval (CI) 2.21–2.29 mM), Gln181 $EC_{50}=1.92$ mM (95% CI 1.81–2.02 mM), Leu341 $EC_{50}=1.99$ mM (95% CI 1.83–2.10 mM). This indicates that these mutations are associated with $G\alpha_{11}$ gain-of-function. Three-dimensional modelling of these mutations predicted conformational changes resulting in reduced stabilisation of the GTP hydrolysis transition state and a reduction in the intrinsic $G\alpha_{11}$ GTPase activity leading to prolonged lifetime of the active GTP-bound $G\alpha_{11}$ subunit. Thus, our results establish a new disorder, ADH type 2 (ADH2) which is due to the first reported germline gain-of-function mutations in $G\alpha_{11}$.

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OC1.8

Kisspeptin advances ovulation in healthy women

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Background
The *KISS1* gene, is a critical regulator of normal reproductive function. In humans, KISS1 deletion results in a failure to go through puberty while activating mutations result in central precocious puberty. Administration of kisspeptin induces ovulation in rodents and sheep. However chronic exposure to exogenous kisspeptin-54 leads to profound tachyphylaxis in women with hypothalamic amenorrhoea. It is not known whether exogenous kisspeptin can alter the menstrual cycle in healthy women.
Aim
To determine the effects of acute and chronic kisspeptin administration on the menstrual cycle in healthy women.
Methods
We performed a prospective, single-blinded, one-way crossover study. Five healthy female volunteers received twice-daily s.c. injections of kisspeptin-54 or saline for 7 days during days 7–14 of their menstrual cycle. All subjects underwent serial assessments of basal reproductive hormones, ultrasound parameters, and LH pulsatility, as well assessment of acute sensitivity to GnRH and kisspeptin injection.
Results
Kisspeptin-54 treatment shortened the menstrual cycle (mean length of menstrual cycle in days: saline 28.6±1.4 vs kisspeptin 26.8±3.1, $P<0.01$), advanced the onset of highest recorded serum LH (mean menstrual day of highest recorded LH: saline 15.2±1.3 vs kisspeptin 13.0±1.9, $P<0.05$), and advanced the onset of the luteal phase of menstrual cycle (mean menstrual day of progesterone increase: saline 18.0±2.1 vs kisspeptin 15.8±0.9, $P<0.05$). On menstrual day 15, the largest ovarian follicle had a significantly higher diameter following kisspeptin-54 when compared with saline (mean diameter of largest follicle (mm): saline 10.0±2.2 vs kisspeptin 15.5±1.2, $P<0.05$). LH pulsatility was maintained during kisspeptin-54 treatment. Sensitivity to exogenous kisspeptin-54 and exogenous GnRH was maintained during twice-daily kisspeptin-54 administration.
Conclusion
We demonstrate for the first time that exogenous kisspeptin-54 advances the menstrual cycle in healthy women. This data has therapeutic implications for the use of kisspeptin in the treatment of women with reproductive disorders.

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Steroids and thyroid

OC2.1

Loss-of-function mutations in IGSF1 cause a novel, X-linked syndrome of central hypothyroidism and testicular enlargement

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Introduction
Congenital central hypothyroidism occurs either as isolated TSH deficiency or in conjunction with other pituitary hormone deficits. Undetected central hypothyroidism is associated with developmental delay in children and adverse cardiometabolic sequelae in adults. Hitherto, mutations in the TRH receptor (TRHR) or TSHβ subunit (TSHB) genes are the only known causes of isolated TSH deficiency.
Methods
Using whole exome and candidate gene sequencing, we have studied 11 unrelated families with males exhibiting isolated TSH deficiency, testicular enlargement and variably low serum prolactin levels.
Results
We have identified eight distinct mutations and two whole gene deletions in the X-linked immunoglobulin superfamily member 1 (IGSF1) gene in affected males. IGSF1 encodes a pituitary-enriched plasma membrane glycoprotein; disease-associated mutations block trafficking of IGSF1 from the endoplasmic reticulum to the membrane, consistent with loss-of-protein function. We have also characterised IGSF1-deficient mice. Adult male IGSF1 null mice show decreased pituitary TSH content and circulating T_3 levels, and increased body weight, recapitulating features of the human disorder. Elevated hypothalamic TRH levels in null mice, in association with decreased pituitary TRHR mRNA levels and blunted serum TSH responses to TRH testing suggests that impaired TRH signalling may be the basis for hypothyroidism.
Conclusions
Collectively, our observations delineate a novel X-linked syndrome in which loss-of-function mutations in IGSF1 cause central hypothyroidism, testicular enlargement and variable prolactin deficiency, and identify a previously unsuspected role for IGSF1 in hypothalamic-pituitary control of thyroid and testicular function.

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OC2.2

Abnormal cardiac bio-energetics in subclinical hypothyroidism; cardiac magnetic resonance spectroscopic study

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Background
It is well established that subclinical hypothyroidism (SCH) is associated with mild ventricular dysfunction and early cardiovascular disease (CVD), but it is unknown if there is an underlying defect in cardiac bio-energetic function.
Objective
To quantify the cardiac phosphocreatine/ATP (PCr/ATP) ratio in SCH, compare with healthy controls (HC) and to measure the effect of 6 months of thyroxine treatment.
Method
Cardiac energetic function (PCr/ATP ratio) was measured using phosphorus-31 magnetic resonance spectroscopy in subjects with SCH (TSH 4.0–10.0 mIU/l, normal free T_4) at baseline and after thyroxine therapy (1.6 cg/kg per day) and compared with age and gender matched HC. All subjects were free of any overt heart disease. 21 subjects with SCH and 17 HC were well matched for age (mean age 39.1 vs 43.3 years), sex (females 80 vs 80%), and mean free T_4 (13.4 vs 14.4 pmol/l) but differed in mean TSH (6.6 vs 2.1 mIU/l, $P<0.001$). A mean serum TSH of 2.0 mIU/l was achieved on treatment with thyroxine.
Results
At baseline PCr/ATP ratio in 21 SCH was lower than in HC (1.8 ± 0.3 vs 2.1 ± 0.2, $P=0.001$). After treatment (data analysed in 16 SCH) PCr/ATP ratio significantly improved (1.9 ± 0.3 vs 1.7 ± 0.2, $P=0.004$) to the level similar to HC ($P=$NS). Serum TSH was similar in HC and SCH post-treatment group ($P=$NS). The PCr/ATP ratio negatively correlated with serum TSH in all subjects ($r=-0.37$, $P=0.026$).
Discussion
To our knowledge, this is the first report demonstrating subnormal cardiac PCr/ATP ratio in SCH subjects and correction by thyroxine treatment. By comparison with studies of overt heart disease and PCr/ATP, our findings in SCH provide further confirmation for the presence of early cardiac impairment in our subjects, which is reversible with thyroxine therapy and provides a rationale for active management of SCH.
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OC2.3

A bi-transgenic murine model of PTTG and PBF overexpression in the thyroid gland

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Whilst the majority of differentiated thyroid cancers (DTC) have oncogenic mutations, a significant minority may be driven by the overexpression of proto-oncogenes. PTTG and PBF are proto-oncogenes which are induced in DTC, elicit tumours in xenograft models and interact *in vitro*, where PBF shuttles PTTG into the nucleus. However, the relative contributions of each gene to DTC has not been delineated. Here, we constructed a bi-transgenic murine model over-expressing both PBF and PTTG specifically in the thyroid gland (PTTG/PBF-Tg), and characterised it in comparison to age and sex matched single transgenic (PTTG-Tg, PBF-Tg) and wild-type mice. A total of 68 wild-type, 98 PBF-Tg, 25 PTTG-Tg and 23 PTTG/PBF-Tg mice were assessed. No significant difference in thyroid weight was observed between male and female mice within each of the four genotypes. However, there was a significant 2.7-fold increase in thyroid weight, adjusted for total body weight, in bi-transgenic mice compared to wild-type ($P<0.001$) at 6 weeks of age. PBF-Tg thyroid weight was 1.7-fold higher than wild-type mice ($P<0.001$), whereas PTTG thyroid weights were similar to wild-type (0.95-fold; $P=0.18$). Interestingly, bi-transgenic thyroids were 1.6-fold heavier than PBF-Tg thyroids ($P<0.001$). Enlarged thyroid growth in bitransgenic mice was accompanied by significant hyperplasia and macro-follicular lesions. As oncogenic expression of PTTG is known to induce genetic instability (GI), we determined GI levels through FISSR-PCR in primary thyroid cultures of each genotype. Compared with WT mice (arbitrary GI index = 0%), PBF-Tg mice had a GI index of $19.8\pm1.8\%$; PTTG-Tg mice of $7.6\pm1.6\%$; and PTTG-Tg/PBF-Tg mice of $37.9\pm2.7\%$. Together, our data reveal a complex interplay between PTTG and its binding partner PBF in vivo; the bi-transgenic thyroid phenotype is closer to that of PBF-Tg mice than PTTG-Tg mice, but reveals increased goitre size and heightened genetic instability than either single transgenic model alone.
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OC2.4

THRA or _DIO2_ mutations are not a common cause of high bone mass in humans

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Mice with dominant-negative mutations of thyroid hormone receptor α1 (TRα1) are euthyroid but display growth retardation and delayed bone age as juveniles and increased bone mass during adulthood, indicating impaired skeletal thyroid hormone responsiveness. The first autosomal dominant mutations affecting TRα1 in humans were recently described in two unrelated children and one parent who were euthyroid apart from a low T_4:T_3 ratio. Consistent with the mouse phenotype both children exhibited skeletal dysplasia and growth retardation but information about the affected parent was limited. The type 2 deiodinase (D2) converts the prohormone T_4 to the active hormone T_3, thus controlling the intracellular supply of T_3 to target tissues. Detailed analysis of D2 knockout mice demonstrated high bone mass and mineralisation resulting from impaired T_3 action in osteoblasts. However, no individuals with mutations affecting D2 have been described.
These data demonstrate that impaired T_3 action in bone results in high bone mineral density (BMD) despite normal circulating thyroid status. Thus, we hypothesised that euthyroid adults with mutations affecting TRα1 or D2 would exhibit high BMD.
We defined a subgroup of 1278 healthy euthyroid postmenopausal women (hip standardised BMD: 863 ± 145; Lumbar spine standardised BMD: 1022 ± 138; mean$\pm$s.d., mg/cm^2; fT_4:fT_3 ratio: 3.58 ± 0.79) from the osteoporosis and ultrasound study (OPUS) population. The *THRA* gene (exons 2-10) was sequenced in 100 subjects with the highest BMD in whom DNA was available ($n=200$ alleles; hip BMD: 1105 ± 112; LS BMD: 1325 ± 156; fT_4:fT_3 ratio: 3.45 ± 0.57) and the *DIO2* gene (exons 1, 2, a, b, SECIS) was sequenced in 48 of these individuals (n = 96 alleles; hip BMD: 1118 ± 123; LS BMD: 1321 ± 159; fT_4:fT_3 ratio: 3.52 ± 0.67).
The T_4:T_3 ratio did not correlate with hip or lumbar spine BMD and no *THRA* or *DIO2* mutations were identified. These data demonstrate that mutations affecting TRα1 or D2 are not a common cause of high bone mass in humans.
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OC2.5

A mutation in thioredoxin reductase 2 (TXNRD2) is associated with a predominantly adrenal phenotype in humans

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Familial glucocorticoid deficiency (FGD, OMIM#202200) is a rare autosomal recessive disorder characterised by adrenal resistance to the action of ACTH, with isolated glucocorticoid deficiency. Recently, mutations in *NNT*, encoding the mitochondrial anti-oxidant nicotinamide nucleotide transhydrogenase have been reported to cause FGD.

Our index case, from a highly consanguineous Kashmiri family, was diagnosed with adrenal insufficiency during a septic episode at the age of 12 years. Her sister was subsequently diagnosed at 4.5 years with a 2 years history of hyperpigmentation. Three of the index case's children were diagnosed with FGD between the ages of 0.3–2.9 years on screening and their first cousin was diagnosed aged 0.1 year after presenting with poor feeding and heart failure secondary to a truncus arteriosis cardiac malformation.

Whole exome sequencing of three affected family members identified a novel homozygous mutation, Y447X in *TXNRD2,* encoding thioredoxin reductase 2 that segregated with the disease in this extended kindred. TXNRD2 is a predominantly mitochondrial selenoprotein, dependent upon a c-terminal selenocysteine residue for reduction of the active site disulphide in anti-oxidant thioredoxins and integral in maintaining thioredoxin activity. *TXNRD2* knockout is embryonic lethal in mice due to cardiac malformation, cardiac specific ablation leads to dilated cardiomyopathy (DCM) and heterozygous mutations have also been described in humans with DCM. The mutation was predicted to lead to premature truncation and removal of the selenocysteine residue, however RT-PCR and western blotting revealed complete absence of TXNRD2 in patients homozygous for the mutation presumably as a result of nonsense-mediated decay of mRNA.

Previous studies describe a delicate balance of mitochondrial redox regulation controlling steroidogenesis at the level of the adrenal gland. We report the first mutation in *TXNRD2* associated with a predominantly adrenal phenotype, indicating the importance of the thioredoxin system in maintaining redox homeostasis in the adrenocortical environment.

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OC2.6

An *N*-ethyl-*N*-nitrosourea induced Corticotrophin releasing hormone promoter mutation provides a mouse model of Cushing's syndrome

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Cushing's syndrome, which is characterised by excessive circulating glucocorticoid (GC) concentrations, may be due to ACTH-dependent or -independent causes that include anterior pituitary and adrenal cortical tumours, respectively. In the course of our phenotype-driven screens of mouse mutants induced by the chemical mutagen *N*-ethyl-*N*-nitrosourea (ENU), we observed a mutant mouse with obesity, hyperglycaemia and low bone mineral density, features that are consistent with Cushing's syndrome. This phenotype was inherited as an autosomal dominant trait and the disease locus was mapped to chromosome 3 and to a 6.6 Mbp interval that contained the gene encoding corticotrophin releasing hormone (*Crh*). DNA sequence analysis of the *Crh* gene did not identify any coding region mutations, in affected mice, but instead a T to C transition at −120 bp relative to the transcription start site, within the *Crh* promoter, was identified. Luciferase reporter assays demonstrated that this T to C transition resulted in a greater than twofold increase in transcription activity in Neuro2a cells ($P<0.001$) and was thus a gain-of-function mutation. $Crh^{-120/+}$ mice, when compared to wild-type littermates ($Crh^{+/+}$mice), had obesity, muscle wasting, thin skin, hair loss, elevated plasma concentrations of corticosterone (mean ± SEM: $Crh^{-120/+} = 721.8 \pm 88.8$ ng/ml; $Crh^{+/+} = 414.3 \pm 59.5$ ng/ml, $P=0.01$) and 24 h urinary concentrations of corticosterone ($Crh^{-120/+} = 504.3 \pm 14.9$ ng/ml; $Crh^{+/+} = 141.9 \pm 30.5$ ng/ml, $P<0.0001$). *In vivo* assessment of $Crh^{-120/+}$ mice revealed them to have elevated plasma concentrations of glucose ($P=0.01$), insulin ($P<0.0001$), leptin ($P<0.01$), cholesterol ($P<0.001$) and triglycerides ($P<0.01$). $Crh^{-120/+}$ mice also had low bone mineral density ($P<0.0001$), hypercalcaemia ($P<0.001$), hypercalciuria ($P<0.0001$) and decreased concentrations of plasma parathyroid hormone ($P<0.05$) and osteocalcin ($P<0.0001$). Thus, a mouse model for Cushing's syndrome has been established and this will help in further elucidating the pathophysiological effects of GC excess.

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OC2.7

11β-hydroxysteroid dehydrogenase type 1: a role in skin wound healing

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Glucocorticoid (GC) excess inhibits wound healing (WH) causing increased patient discomfort and infection risk. The GC-activating enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) regulates local GC availability in tissues including liver, adipose, and muscle. 11β-HSD1 is also expressed in skin, where studies recently demonstrated increased levels in older donors and a reversal of age-induced dermal atrophy in 11β-HSD1-null mice. However, the role of 11β-HSD1 during WH remains to be elucidated.

Following ethical approval, two 5 mm full-thickness dorsal wounds were generated in female SKH1-HR mice and collected on day 0 (d0), d2, d4, d8 ($n=4$). 11β-HSD1, cofactor-supplying hexose-6-phosphate dehydrogenase (H6PDH), glucocorticoid receptor (GR) and the differentiation marker filaggrin were analyzed by qPCR (normalized to 18S rRNA). 11β-HSD1 protein was analyzed by Western blot in dispase-separated epidermis/dermis ($n=4$, normalized with β-actin). Confluent primary mouse keratinocytes were differentiated for 48 h with 1.5 mM calcium ± 200 nM corticosterone ± the GR inhibitor RU486 ($n=3$ each group).

11β-HSD1 protein increased substantially in full-thickness wounds at d2 relative to d0 and d2 unwounded skin (negligible), was reduced at d4 (detectable exclusively in wounded dermis), and was negligible by d8; qPCR revealed a 14-fold mRNA increase at d2 (vs unwounded, $P<0.05$), fourfold increase at d4 ($P<0.05$) decreasing to baseline levels by d8. H6PDH mRNA increased in d4 and d8 wounds (twofold, $P<0.05$) whilst filaggrin mRNA increased in d8 wounds (1.6-fold, $P<0.05$). GR mRNA levels were unaffected by wounding.

Increased 11β-HSD1 during early WH suggests GC activation could potentiate subsequent keratinocyte differentiation. Indeed, calcium-treated keratinocytes displayed increased filaggrin and H6PDH mRNA only during GC co-incubation (6- and 12-fold respectively $P<0.01$), effects blocked by RU486. GR expression was unaffected by differentiation.

Therefore, 11β-HSD1 blockade may accelerate WH by limiting keratinocyte differentiation, promoting proliferation/migration and re-epithelialization, of potential importance in older patients exhibiting elevated basal 11β-HSD1 levels and impaired WH.

Declaration of funding

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OC2.8

When math meets biology: systems approach to drug resistance analysis

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Glucocorticoids (GCs) have an important role in inflammation, apoptosis and immunosuppression and are among the most widely prescribed medications in clinical practice. GCs exert their effect by binding to the transcription factor, glucocorticoid receptor (GR). GCs are used in the treatment of acute lymphoblastic leukaemia (ALL) as they induce apoptosis in lymphoid cells, however resistance and side effects still occur frequently. Computational modeling has enormous potential in the understanding of biological processes such as apoptosis and the discovery of novel regulatory mechanisms.With the advances in high-throughput technology, vast amount of 'omics' type of data make the study of drug resistance challenging. Here we use systems biology with the ultimate goal of increasing understanding of GR function and predicting future experimental approaches. As Bcl-2 family of genes that control apoptosis is a key determinant of GC function in ALL, we built kinetic models based on ordinary differential equations that facilitated investigation of the molecular mechanisms of GCs mediated Bim and Bmf induction. To gain a global view on GR resistance in ALL and to extend the previously established models, we performed integrated timecourse microarray analysis in ALL cell lines and clinical samples. This approach identified c-Jun and Erg as crucial determinants of GC resistance and demonstrated that using Erg inhibitors increased apoptosis of ALL cells. Finally, adopting variety of genomewide experimental study designs coupled with specific clustering analysis, we demonstrate that stem cells and bone marrow microenvironment alter expression profiles of genes that control signalling, apoptosis, autophagy and inflammation and increase ALL chemoresistance.

In conclusion, our findings represent a successful example of utilising systems biology to study causes of drug resistance. These approaches aid discovery of

biomarkers of GC resistance, advance our understanding of drug sensitivity, link host-tumour interactions to chemoresistance and can be used to improve therapy of leukemia.
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Reproduction, growth and Development

OC3.1

Identification of very early sorting endosomes that spatially program gonadotrophin hormone receptor signalling

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G protein-coupled receptors (GPCRs) are one of the largest families of the mammalian genome and represent the single most common therapeutic target. The glycoprotein hormones; LH and human chorionic gonadotrophin, act at a single GPCR the LH/hCGR, whose roles in reproductive function and pregnancy are well known. Endocytic trafficking of GPCRs represents a key mechanism in defining cellular responses in complex signaling pathways by controlling both the temporal and spatial parameters of cellular signalling. Studies on LH/hCGR signalling have unveiled unexpected and diverse facets to its regulation via endocytic trafficking. The early endosome (EE) has traditionally been thought of as the earliest primary site of protein sorting in the endocytic pathway. Using the LH/hCGR as a model receptor, we identify a biochemically distinct compartment, preceding the EE that mediates the post-endocytic sorting of the LH/hCGR. We show that these very early sorting endosomes (VESEs) contain the adaptor protein APPL1 (adapter protein containing PH domain, PTB domain and leucine zipper motif) yet do not contain the EE markers Rab5 and EEA1. Receptors are directed to and sorted from this compartment to the regulated recycling pathway, through interactions with the PDZ protein GAIP-interacting protein (GIPC, C terminus). The C-terminal tail of the LH/hCGR was both necessary and sufficient for interacting with GIPC and targeting to the VESE. Loss of cellular GIPC, via siRNA-mediated knockdown, reroutes receptors to EEs and prevents sorting to the recycling pathway. Total-internal reflection microscopy revealed that GIPC is recruited to LH/hCGR in clathrin-coated pits. Importantly, altering the trafficking of receptors from the VESEs to the EEs, or enriching cargo in the VESE reprograms LH/hCGR signalling. These findings reveal an unprecedented heterogeneity in the endocytic platforms involved in cargo sorting. Further, that endosomal signalling from VESEs provides acute spatial compartmentalization for potentially diverse receptor signalling systems.
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OC3.2

Heterodimerisation of GNRH receptors modifies the LH-induced calcium signalling profile

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The gonadotrophin receptors, LH receptor (LHR) and FSHR are G-protein coupled receptors, vital in mediating reproductive functions. During the follicular phase of the ovarian cycle, FSHR and LHR are separately localised to discrete cellular compartments, granulosa and theca cells respectively, where they control steroidogenesis and follicle maturation. However, as the follicle develops, LHR expression is induced in granulosa cells, resulting in co-expression of FSHR and LHR in a single cellular compartment. Remarkably, little is known about the functional significance of this co-expression. While both FSHR and LHR are known to homodimerise, the question of whether FSHR and LHR can form functional heterodimers remains to be explored. Therefore, this study aims to determine if FSHR and LHR can form heterodimers and assess the functional impact of such heterodimer formation. The ability of FSHR and LHR to heterodimerise in live, intact cells was observed through the use of bioluminescence resonance energy transfer. The ability of the heterodimer to impact receptor cell surface expression showed no significant effects on cell surface trafficking of either receptor. Further, Gαs/cAMP signalling was not altered in the LHR/FSHR heterodimer compared to cells expressing each receptor alone. Interestingly, the pattern of LHR-induced Gαq/calcium signalling was significantly altered in the presence of FSHR, from an acute and rapid signal to a more sustained calcium response. The prolonged calcium signal from LH activated LHR/FSHR expressing cells appears to be mediated through activation of L-type calcium channels. Use of a Gαi inhibitor, pertussis toxin, had no effect on calcium signalling indicating there may be no alteration in G protein-coupling of the heterodimer. The mechanisms underlying this change in calcium signalling patterns will be further assessed. Overall this study indicates that LHR/FSHR heterodimers may represent a key mechanism for generating sustained calcium responses in preovulatory follicles.
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OC3.3

Regulation of G protein-coupling specificity via *cis* and *trans* activation of the LH/chorionic gonadotrophin receptor (LHCGR)

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Accepted dogma once stated that G protein-coupled receptors (GPCRs) function as monomers, however, over the last decade *in vitro* experiments have shown GPCRs to function as dimers and higher order oligomers. We have recently reported the first *in vivo* evidence for the physiological importance of Class A GPCR homodimerisation using the LHCGR as a model receptor. Transgenic co-expression of binding deficient LHCGR ($LHCGR^{-LH}$) and signalling deficient LHCGR ($LHCGR^{-cAMP}$) reversed the hypogonadism and infertility of male LHCGR null mice. Utilising the $LHCGR^{-LH}$ and $LHCGR^{-cAMP}$ as tools for studying *cis* (same receptor binding hormone and propagating signal) and *trans* (one receptor partner binding hormone, and one propagating signal) activation through receptor dimerisation, we aimed to interrogate whether these distinct modes of receptor signalling result in ligand bias to a receptor that can couple to multiple G proteins, using the endogenous ligands, LH and hCG. In cells expressing either WT LHCGR or $LHCGR^{-LH}$/$LHCGR^{-cAMP}$, hCG and LH showed equivalent Gαs- cAMP responses indicating that *trans*-activation was sufficient to mediate the Gαs response to both ligands. hCG-dependent Gαq signalling was comparable in WT LHCGR and $LHCGR^{-LH}$/$LHCGR^{-cAMP}$ expressing cells. However, LH-dependent Gαq signalling was severely diminished in the *trans*-activation mode of signalling, revealing a requirement of *cis*-activation for full LH-dependent Gαq signalling. Assessment of ligand induced receptor-G protein interactions by bioluminescence energy transfer (BRET) also showed equivalent hCG and LH-dependent Gαs coupling, however LH-dependent Gαq receptor-association in the transactivation model was diminished. Interestingly, varying the surface expression of the $LHCGR^{-LH}$ to the $LHCGR^{-cAMP}$ indicated that *trans*-activation to Gαs signalling favours an oligomeric complex containing an excess of $LHCGR^{-cAMP}$. This study indicates that *cis*- and *trans*- modes of GPCR activation can result in ligand-induced diversification of signalling, and may provide mechanistic insight into how GPCR dimers/oligomers, via *trans*-activation, can regulate the degree of activity of G-protein signalling.
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OC3.4

Characterising changes in the *in vivo* male rodent brain using magnetic resonance spectroscopy

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Background
By providing a non-invasive, functional insight into brain chemistry, MRS has the

potential to provide objective longitudinal data on mammalian brain development.
Aims
To assess the sexual dimorphism in rodent brain chemistry and development using *in vivo* MRS.
Methods
26 (19 males) Sprague–Dawley rats were scanned at 6 weeks and 12 male rats at 10 weeks using a 7T MRI scanner. Testosterone concentrations were measured by ELISA and reproductive organs and brain tissue collected. Metabolites were expressed as a ratio to creatine and full width at half-maximum (FWHM) of the water peak was used as a guide to the reliability of the ratios.
Results
Median weight in 6 wk males, 6 wk females and 10 wk males was 197 g (142–230), 131 g (121–135) and 316 g (274–365) respectively. Median anogenital distance (AGD) in 6 wk males, 6 wk females and 10 wk males was 2.46 cm (1.89–2.9), 1.17 cm (1.04–1.19) and 3.25 cm (2.8–3.6). Median male serum testosterone at 6 and 10 weeks were 1.33 ng/ml (0.23–5.45) and 3.36 ng/ml (1.75–8.26). Median brain weight at 6 weeks in three male and three female rats was 1.84 g (1.8–1.84) and 1.78 g (1.75–1.8). Median prostate weight at 6 and 10 weeks in 3 and 12 male rats was 0.22 g (0.20–0.47) and 0.56 g (0.44–0.76). Median testes weight at 6 weeks was 0.83 g (0.8–0.94). Median phallus weight at 6 weeks was 0.12 g (0.11–0.14). 14 metabolites were identified in the occipitofrontal cortex. FWHM range was 12–38 Hz. In the 6 wk male rats, myo-inositol ratios showed a positive association with testosterone levels ($P=0.04$), and AGD was correlated with phosphocreatine ($P=0.033$) and glutamate ($P=0.045$). In addition, there was an increase from 6 to 10 weeks in three metabolite ratios: taurine ($P=0.025$), myo-inositol ($P=0.012$) and phosphocholine ($P=0.005$).
Conclusions
MRS is a reliable tool for studying the brain in maturing rats and may be a useful tool for studying the link between longitudinal changes in sex steroids and brain development.
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OC3.5

Effects of *in utero* exposure to acetaminophen (paracetamol) on steroidogenesis by the rat and human fetal testis

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Reproductive disorders in males that manifest at birth (cryptorchidism, hypospadias), or young adulthood (low sperm count, lower testosterone levels, testicular cancer) may share a common origin and constitute a 'testicular dysgenesis syndrome' (TDS). Normal reproductive tract development is programmed by androgens within the 'masculinisation programming window' (MPW, e15.5–e18.5-rats; ~8–14 weeks' gestation-humans). In rats, TDS disorders can arise because of deficiencies in this programming. Epidemiological studies have shown that acetaminophen (paracetamol) use by women during pregnancy during the presumptive MPW is associated with increased risk of cryptorchidism in boys at birth. Furthermore, exposure of rats to acetaminophen has been shown to reduce testosterone production by the fetal testis and to cause a small significant reduction in anogenital distance (AGD, which provides a read-out of testosterone production/action during the MPW). We investigated the effects of acetaminophen exposure (350 mg/kg per day) on fetal rat masculinisation and on human fetal testis testosterone production using an *ex-vivo* xenograft system. *In utero* exposure of pregnant rats to acetaminophen increased bodyweight postnatally, but not in fetal life. Intratesticular testosterone was significantly reduced at embryonic day (e)17.5 after acetaminophen exposure, which accounted for a small but significant reduction in AGD at e21.5; AGD was unaffected in pubertal and adult rats. No effects on postnatal testis weight, penis length, and occurrence of hypospadias or cryptorchidism were observed in rats exposed *in utero* to acetaminophen (although offspring number studied was low). Similar acetaminophen exposure (1 week) of castrate nude mice bearing human fetal testis xenografts non-significantly lowered blood testosterone levels, but significantly reduced host seminal vesicle weight. In conclusion, gestational exposure to acetaminophen causes a mild reduction in fetal testosterone production in the rat and human fetal testis, which may explain the increased risk of cryptorchidism reported after maternal use of acetaminophen in late 1st/early 2nd trimester of pregnancy.
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OC3.6

Follistatin-like 3 (FSTL3), a transforming growth factor β ligand inhibitor, is essential for placental development in mice

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Follistatin-like 3 (FSTL3) is an endogenous glycoprotein inhibitor of transforming growth factor-β (TGFβ) ligands such as activin. TGFβ ligands are integral to key cellular processes such as proliferation, development and differentiation, including angiogenesis. It is not clear, however, how FSTL3 and activin action affect tissue and organ functions in health and disease. We have identified a group of activin-responsive genes that have an expression pattern closely aligned to that of FSTL3 and our expression analyses support the likelihood that FSTL3 action is important in cardiovascular tissues and potentially angiogenesis. To test the hypothesis that FSTL3 is indeed a regulator of angiogenesis we studied the placenta in FSTL3 gene deleted mice (FSTL3 KO). Our findings reveal significant defects in the FSTL3 KO placenta when compared to WT. Gross overall analysis showed a significant increase in size at 16.5 and 18.5 dpc compared to WT. Concomitantly, placental efficiency was significantly reduced at 18.5 dpc. While gross morphology is altered from flat to domed in shape, histology and immunohistochemistry reveal morphological differences in placental junctional zones in FSTL3 KO placenta compared to WT. Most strikingly, the FSTL3 KO placenta has significantly reduced red blood cell amount strongly supportive of a role for FSTL3 in angiogenesis. Finally, activin responsive FSTL3-synexpression genes are upregulated in FSTL3 KO placenta. Thus we conclude that FSTL3 function is crucial for normal placental development and function and that the FSTL3 synexpression genes identified might contribute to an activin-responsive effector network important in normal placental development.
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OC3.7

Disruption of mesenchymal glucocorticoid signaling attenuates embryonic lung development and results in post natal lethality in mice

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Glucocorticoid signalling is essential during embryonic lung development, with both the global and epithelial glucocorticoid receptor (GR) null mice presenting with lung atelectasis and post natal lethality. In this study, we examined the role of glucocorticoid signalling within mesenchymal tissues. To study the role of the GR in mesenchymal tissues during embryogenesis we crossed GRflox mice with Dermo1-Cre mice to generate GR^{Dermo1} mice, where the GR gene was conditionally deleted within mesenchymal cells. Organ development between E14.5 through to birth was determined by histological staining and MRI performed at E18.5. Specific mesenchymal cell populations were assessed by immunohistochemistry and quantitative RT-PCR. GR^{Dermo1} mice displayed severe pulmonary atelectasis, defective abdominal wall formation and postnatal lethality. GR^{Dermo1} mice failed to progress from the canalicular to saccular stage of lung development, evidenced by the presence of immature air sacs, thickened interstitial mesenchyme and an underdeveloped vascular network between E14.5 and E18.5 (lung tissue to alveolar space; GR^{Dermo1}, 90.3% vs WT, 79.6%, $P<0.001$). Interstitial fibroblast numbers were expanded within GR^{Dermo1} mice compared to WT littermates (50.1 vs 21.1 cells/ 50 μm^2; $P<0.005$). However, myofibroblasts, vascular smooth muscle and endothelial cells were shown to be present in normal numbers. Analysis of their functionality revealed that myofibroblasts from GR^{Dermo1} mice possessed significantly reduced elastin synthesis. In contrast epithelial lining cells of immature saccules were poorly differentiated. Reduced elastin and collagen deposits were also noted in connective tissues adjacent to the umbilical hernia. This study demonstrates that eliminating the GR in cells of the mesenchymal lineage results in marked effects on interstitial fibroblast function, including a significant decrease in elastin synthesis. This results in lung atelectasis and postnatal lethality, as well as additional and hitherto unrecognized developmental defects in abdominal wall formation. In addition, altered glucocorticoid signalling in the mesenchyme indirectly attenuates normal lung epithelial differentiation.
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OC3.8

Adiponectin induces GSK3 kinase-mediated cross-tolerance to endotoxin in macrophages

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Adiponectin, an exclusive adipose-derived hormone, circulates at high concentration, and exerts metabolic effects. Adiponectin levels in the circulation correlate negatively with BMI, and low adiponectin concentrations are associated with the low grade inflammation and metabolic dysfunction that accompanies obesity. Adiponectin has been reported to have potent anti-inflammatory activities, and to exert these effects by regulating macrophage function. The mechanism of adiponectin action remains unclear, but a broad effect on expression of pro-inflammatory cytokines has been suggested. Here, we show that prior exposure of primary murine macrophages to adiponectin for as little as 3 h was sufficient to render them tolerant to subsequent exposure to lipopolysaccharide (LPS), with grossly reduced proinflammatory cytokine output. Continued exposure to adiponectin was required to maintain tolerance, with macrophages regaining sensitivity to LPS one day following wash out of adiponectin. Adiponectin-induced cross-tolerisation was mediated through suppression of Toll-like receptor signalling, specifically via induction of negative feedback by the signalling inhibitor A20. Further, adiponectin-induced cross-tolerance was dependent on the kinase GSK3, which was required for induction of A20. Pretreatment with the GSK3 inhibitor SB216763 attenuated adiponectin-induced A20 expression and blocked adiponectin-induced cross-tolerance. Our data suggest that adiponectin's constant presence in the circulation at high levels (in lean subjects) renders macrophages resistant to pro-inflammatory stimuli for the prevention of prolonged and excessive inflammation.

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Obesity, metabolism and bone

OC4.1

Glucocorticoid receptor deficiency in cardiomyocytes causes pathological cardiac remodelling in mice

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Variation in the glucocorticoid receptor (GR) gene associates with relative glucocorticoid resistance, hypertension and increased cardiovascular disease risk in humans. To investigate the contribution of cardiac GR to this phenotype we have characterised adult male mice with cardiomyocyte and vascular smooth muscle deletion of GR (SMGRKO) and have found left ventricular function to be impaired.

SMGRKO mice, generated by crossing GR 'floxed' mice (congenic on C57BL/6J) with SM22α-Cre mice, have reduced cardiac GR protein and mRNA levels (by 52 and 57%, respectively), compared to Cre-negative littermate controls.

The Visualsonics Vevo 770 High-Resolution Ultrasound *In Vivo* Micro-Imaging System was used to assess cardiac function at 10 weeks of age. Mitral valve Doppler showed a detrimental increase in the myocardial performance index (MPI), a marker of combined systolic and diastolic function, in SMGRKO mice. This was primarily due to greater isovolumetric contraction time (control: 12.9 ± 0.8 ms, SMGRKO: 15.5 ± 0.6, $P<0.05$) indicating impairment of the initial left ventricular contractile phase.

Heart weight (% body weight) is increased in SMGRKO mice (control: 0.5 ± 0.02%, SMGRKO: 0.55 ± 0.01%, $P<0.05$) and they have elevated levels of cardiac mRNA encoding myosin heavy chain-β (control: 100 ± 9%, SMGRKO: 151 ± 16%, $P<0.05$), mineralocorticoid receptor (MR (control: 100 ± 14%, SMGRKO: 151 ± 19%, $P<0.05$)) and pro-fibrotic factors (collagen, TGFβ?, CTGF). Cardiac expression of Ca^{2+} handling genes was unaltered. Histopathology shows fibrosis and a trend for increased cardiomyocyte cross sectional area in the left ventricle of SMGRKO mice, suggestive of cardiomyocyte hypertrophy and pathological remodelling despite normal blood pressure.

These data demonstrate that cardiomyocyte/smooth muscle GR deficiency causes pathological changes in the left ventricle resulting in impairment of isovolumetric contraction in 10 week old mice. The findings support a role for cardiomyocyte GR in determination of cardiovascular disease risk.

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OC4.2

11β-HSD1 knockout mice are protected from the adverse metabolic effects of exogenous glucocorticoid excess

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Glucocorticoids (GC), such as prednisolone, are widely prescribed for their anti-inflammatory and immunosuppressive properties. However, they have significant side-effects including insulin resistance and hepatic steatosis. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) converts 11-dehydrocorticosterone (11DHC) to active corticosterone (CORT) and thus amplifies local GC action. We hypothesise that enhanced local GC regeneration of exogenously administered GCs by 11β-HSD1 may contribute to the adverse side-effect profile. To test this hypothesis, 6-week-old male 11β-HSD1−/− and wildtype (WT) mice were treated with CORT (100 μg/ml), 11DHC (100 μg/ml) or vehicle via the drinking water. After 5 weeks, animals underwent glucose tolerance testing and were sacrificed for assessment of metabolic parameters. As anticipated, 11DHC treated 11β-HSD1−/− mice were indistinguishable from vehicle treated mice. CORT and 11DHC treated WT mice displayed impaired glucose tolerance, hepatic steatosis and increased hepatic expression of the fatty acid transporter CD36. However, 11β-HSD1−/− CORT treated mice were protected from glucose intolerance, hepatic steatosis and had lower hepatic CD36 expression. In CORT treated WT adipose tissue, 11β-HSD1 and hormone sensitive lipase (HSL) expression were elevated, associated with increased circulating free fatty acid (FFA) levels. Conversely, CORT treated 11β-HSD1−/− mice were protected from elevated HSL expression and increased circulating FFAs. Finally, intramyocellular diacylglyceride (DAG) content was elevated in CORT and 11DHC treated WT mice, whereas CORT treated 11β-HSD1−/− mice were protected from increased DAG levels, a possible factor in improved glucose tolerance. Importantly, both WT and 11β-HSD1−/− CORT treated mice had a similar increased circulating CORT (500 nmol/ml) compared to vehicle controls (50 nmol/ml). These data demonstrate that 11β-HSD1−/− mice are protected from the adverse effects of exogenous GC excess, and suggest that local GC regeneration may contribute significantly to the adverse effect profile of therapeutic GC use. This raises the possibility of using selective 11β-HSD1 inhibitors as an adjunctive therapy to limit the side-effects of GC treatment.

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OC4.3

Adult offspring of undernourished sheep exhibit epigenetic alterations in HPA axis glucocorticoid receptor

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Maternal programming increases the risk of alterations in the offspring's HPA axis. Previously we showed that maternal undernutrition in sheep induces epigenetic changes in the glucocorticoid receptors (GR) within hypothalamic energy balance pathways, without affecting HPA axis GR. However, these studies focussed on fetal tissues[1]. Here, we investigated whether GR is epigenetically altered in the HPA axis of adult offspring to determine the status of the pathways in adult life.

Ewes were maternally undernourished from 60 days before until 30 days after conception (UN) or fed *ad libitum* (N). Term = 148 days. Brain tissue from adult offspring (mean age 4.4 years) were collected for analysis.

Hippocampal GR mRNA expression was decreased in female and male UN offspring (27% ($P<0.03$) and 28% ($P<0.02$) respectively) compared with controls. Similarly, GR protein levels were decreased in UN offspring (UN

females; 64%, ($P<0.02$): UN males 40% ($P<0.004$)). Epigenetic analysis found a 50% increase in GR promoter methylation in UN females ($P<0.02$) but not in males. However, changes in epigenetic histone markers (H3K9 acetylation and H3K27 trimethylation) associated with the GR promoter were found in both males and females, consistent with GR expression data.

Within the pituitary, epigenetic changes in GR were gender specific. These were associated with decreased GR mRNA and protein in UN females (mRNA: 39% ($P<0.01$); protein: 43% ($P<0.04$)) and increased GR mRNA and protein in UN males (mRNA: 94% ($P<0.05$); protein: 118% ($P<0.004$)) compared with controls.

These studies in adult offspring find changes in the epigenetic and expression status of GR in the HPA axis not found at the fetal stage that were both tissue and gender specific. Therefore, maternal undernourishment during pregnancy results in alterations in the HPA axis which are manifest in adult sheep, suggesting plasticity in the effects of programming.

1. Stevens *et al*. *Endocrinology* 2010 **8** 3652–3664.

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OC4.4

Cholestatic pregnancy programmes metabolic disease in the offspring

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Epidemiological studies have identified the intrauterine environment as a major contributor to increased rates of metabolic disease in adults, but the underlying mechanisms are poorly understood. Intrahepatic cholestasis of pregnancy (ICP) is a common liver disease of pregnancy that affects 0.5–2% pregnant women and is characterised by increased bile acid (BA) levels in the maternal serum. The influence of ICP on the metabolic health of offspring is unknown.

We analysed the North Finland birth cohort (NFBC) 1985/86 database and found that 16-year-old children of mothers with ICP had altered lipid profiles and increased BMI compared to the offspring of uncomplicated pregnancies. We investigated the effect of maternal cholestasis on the metabolism of adult offspring by using a mouse model of gestational cholestasis. The 18-week-old females from cholestatic mothers developed a severe obese, diabetic phenotype with hepatosteatosis following western diet (WD) feeding for 6 weeks compared to mice not exposed to cholestasis *in utero*. Female littermates were susceptible to metabolic disease prior to dietary challenge, as indicated by a pro-inflammatory profile, mild hepatosteatosis and elevated serum adipocytokines. In human and mouse placentas, we demonstrated that gestational cholestasis causes accumulation of lipids. We showed increased transplacental cholesterol transport and *de novo* fetal hepatic lipid synthesis in cholestatic pregnancy. Furthermore, maternal cholestasis in the *Agouti viable yellow* (A^{vy}) mouse model altered the epigenome of the offspring.

This is the first report showing that maternal cholestasis in the absence of altered maternal BMI or diabetes can cause metabolic disease in the offspring. We have demonstrated that the offspring phenotype is programmed by epigenetic alterations and also impaired lipid transport as a consequence of maternal hypercholanaemia.

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OC4.5

Energy intake following infusion of glucagon and GLP-1: a double-blind crossover study

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Obesity is a growing global epidemic and current medical therapies have proven inadequate. Endogenous satiety hormones provide an attractive target for the development of drugs which aim to cause effective weight loss with minimal side effects. Two related peptide hormones, glucagon and glucagon-like peptide 1 (GLP-1), are the subject of this investigation. Both have been found to reduce appetite and cause weight loss. Additionally, glucagon increases energy expenditure. It is proposed that co-administration of both peptides will have an additive effect on appetite reduction, while GLP-1 will protect against the hyperglycaemic effect of glucagon.

In this double-blind crossover study, a weight-adjusted dose of each peptide, alone or in combination, or placebo, was infused into 12 human volunteers for 120 min. An *ad libitum* meal was provided after 90 min and calorie intake determined. Resting energy expenditure was measured by indirect calorimetry at baseline and during the infusion. At regular time points blood samples were taken for assay of glucose, insulin, GLP-1 and glucagon. Pulse, blood pressure and self-perceived nausea levels were also recorded at each time point.

Co-infusion of glucagon with GLP-1 led to a reduction in food intake of 17.9%. Furthermore, the addition of GLP-1 protected against glucagon-induced hyperglycaemia and a trend of increased energy expenditure was seen on co-infusion of glucagon with GLP-1. This was achieved in the absence of negative effects on cardiovascular parameters.

This study therefore supports the concept of GLP-1 and glucagon dual agonism as a possible treatment for obesity.

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OC4.6

Transgenic disruption of 5α-reductase 1 increases susceptibility to liver fibrosis

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5α-Reductase 1 (5aR1) catalyses A-ring reduction of glucocorticoids and androgens, predominantly in liver and modulates steroid hormone action. We previously demonstrated transgenic disruption of 5aR1 predisposes mice to developing fatty liver. Here we tested whether 5aR1 disruption increases susceptibility to the development of liver injury, using the carbon tetrachloride induced liver fibrosis model.

Male 5aR1−/− (KO) mice and wild-type controls (WT) were studied aged 4–5 months following 6 weeks of twice-weekly injection of carbon tetrachloride (CCl_4; 0.3 μl/g) or vehicle (olive oil); $n=4-8$/group. Liver fibrosis was assessed by picrosirius red staining (PSR) of collagen in fixed liver sections and quantified by pixel counting. Plasma and liver triglycerides were measured colourimetrically and liver RNA transcripts quantified by real-time PCR. Data are mean ± S.E.M.; $^*P<0.05$.

Body weight was not different between WT and KO mice, and was not altered by 6 weeks CCl_4 treatment. However, collagen deposition stimulated by CCl_4 treatment was more marked in KO than WT (6575 ± 873 vs 4776 ± 398 pixels*). Liver weight was not different between WT and KO, but triglyceride content was higher in KO (24.5 ± 2.3 vs 16.1 ± 1.6 nmol/mg*). CCl_4 did not alter liver weight or triglyceride content in WT, but reduced liver weight (19%*) and depleted liver triglyceride (60%*) in KO. Plasma triglyceride concentration was correspondingly increased in CCl_4 treated KO (80% vs KO-vehicle* and 43% vs WT- CCl_4*). CCl_4 increased RNA for pro-fibrotic α-SMA and TIMP-1 (12-* and 3-fold* respectively), but there was no differential effect between WT and KO.

In summary, disruption of 5aR1 is associated with fatty liver and increased susceptibility to liver injury in the CCl4 induced liver fibrosis model. This more severe injury is associated with depletion of liver triglycerides coupled with a rise in circulating triglycerides. The mechanism underpinning the increased injury is not clear, but may have important implications for patients being treated with 5aR inhibitors.

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OC4.7

Familial hypocalciuric hypercalcaemia type 3 is caused by mutations in adaptor protein 2 sigma 1

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Familial hypocalciuric hypercalcaemia (FHH) is an autosomal dominant disorder characterized by lifelong elevation of serum calcium concentrations with inappropriately low urinary calcium excretion. Three types referred to as FHH1, FHH2 and FHH3 and located on chromosomes 3q21.1, 19p and 19q13.3, respectively, have been reported. FHH1, caused by loss-of-function mutations of the calcium-sensing receptor (*CaSR*), accounts for >65% of FHH patients. To identify the genetic defect in FHH3, we performed exome sequencing in patients from two unrelated FHH3 kindreds. This revealed a C to T transition, that predicted occurrence of an Arg15Cys missense mutation, in adaptor protein 2 sigma 1 (*AP2S1*), encoding AP2σ2, which was demonstrated to co-segregate with FHH3 in 32 affected members from five generations of the two kindreds. This mutation is predicted to alter an evolutionary conserved arginine residue. To determine the frequency of *AP2S1* mutations in the ~35% of FHH patients without *CaSR* mutations, we undertook DNA sequence analysis of *AP2S1* in 50 additional unrelated patients. This revealed occurrence of 11 missense heterozygous mutations, consistent with autosomal dominant inheritance of FHH3, that all affected Arg15 and consisted of four Arg15Cys, three Arg15His, and four Arg15Leu mutations. Wild-type and mutant AP2σ2 were transiently expressed in HEK293 cells, stably transfected with CaSR, and assessed for their response to changes in extracellular calcium levels. This demonstrated that mutant AP2σ2 decreased the sensitivity of these cells to extracellular calcium and reduced CaSR endocytosis. AP2σ2 forms part of the AP2 complex that has a role in G protein-coupled receptor recycling, and examination of the crystal structure of AP2 revealed that replacement of the Arg15 residue of AP2σ2 compromises a key contact with acidic dileucine motifs of cargo proteins. Thus, our studies have identified the genetic defect underlying FHH3, and give important insights into calcium homeostasis.

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OC4.8

Peptide YY regulates bone mineral content and strength

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Bone loss in anorexia nervosa and following bariatric surgery is associated with an elevated circulating concentration of the gastrointestinal anorexigenic hormone peptide YY (PYY), which acts principally via the Y1R and Y2R receptors. Selective deletion of Y1R in osteoblasts or Y2R in the hypothalamus results in high bone mass, but deletion of PYY has resulted in conflicting skeletal phenotypes leading to uncertainty regarding its role in the regulation of bone mass. We hypothesised that PYY is a negative regulator of bone turnover and strength and determined the consequences of PYY deletion in knockout mice.
We investigated the skeletal phenotype of PYYKO mice during growth (postnatal day P14) and adulthood (P70 and P186) using X-ray microradiography, micro-CT, backscattered electron scanning electron microscopy (BSE-SEM), histology, histomorphometry and mechanical testing.
Long bones from adult PYYKO mice were stronger (maximum load: WT 9.59±5.3N, PYYKO 12.67±3.1N, $P<0.001$, $n=7$–8), more rigid (Stiffness: WT 28.98±1.65 N/mm, PYYKO 36.06±1.51 N/mm, $P<0.01$, $n=7$–8) and tougher (energy dissipated prior to fracture: WT 54.57±4.73%, PYYKO 82±4%, $P<0.05$, $n=7$–8;) than WT controls and displayed increased cortical thickness (WT 0.19±0.007 mm, PYYKO 0.22±0.004 mm, $P<0.01$, $n=7$–8) and mineral content ($P<0.001$, $n=7$–8). Investigation of the mechanisms responsible revealed the phenotype did not result from altered skeletal development or reduced osteoclastic bone resorption, thus suggesting enhanced osteoblastic bone formation as the underlying cause.
These data are consistent with a role for PYY as a negative regulator of bone formation that mediates the detrimental skeletal consequences of anorectic conditions such as starvation, malignancy and cardiac failure.

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Pituitary and Neoplasia

OC5.1

Genetic background influences tumour phenotype in heterozygous *Men1* knockout mice

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Multiple endocrine neoplasia type 1 (MEN1), an autosomal dominant disorder characterised by the occurrence of parathyroid, pancreatic islet and anterior pituitary tumours, is due to mutations of a tumour suppressor gene, *MEN1*. *MEN1* mutations have also been reported to cause familial isolated primary hyperparathyroidism (FIHP). Moreover, 15 identical *MEN1* mutations have been reported to cause MEN1 or FIHP in unrelated families; thereby implicating a role for genetic modifiers in the expression of the *MEN1* mutation. To elucidate the role of genetic modifiers, we utilised a mouse knockout model for MEN1 in which <90% of heterozygous mice, lacking one *Men1* allele that has exons 1 and 2 deleted ($Men1^{+/-}$), develop parathyroid, pancreatic islet and anterior pituitary tumours, by the age of 18 months. $Men1^{+/-}$ mice were backcrossed for 10-18 generations to produce two congenic strains that had ≥99.9% genetic identity to either the C57BL/6 or 129S6/SvEv strain, and the pituitaries and pancreata and were examined for tumour development in 207 $Men1^{+/-}$ mice (81 males; 126 females), aged 18-26 months. This revealed that the frequency of pituitary tumours was influenced by the background strain. Thus, in female $Men1^{+/-}$ mice aged over 18 months, anterior pituitary tumours (expressing prolactin and growth hormone) were significantly more frequent in C57BL/6 than in 129S6/SvEv mice (78.8 vs 38.3% respectively; $P<0.005$); whereas, the frequency of pancreatic islet cell tumours in the two strains was similar (males 90.2 vs 97.5% and females 95.0 vs 87.9%; $P>0.2$). However, immunohistochemical examination of pancreatic islet tumours ($n=25$) revealed that glucagon-immunostaining tumours developed significantly more frequently in the 129S6/SvEv than the C57BL/6 strain (70.0 vs 6.7% respectively; $P<0.002$). Thus, our results demonstrate that genetic modifiers in the two mouse strains, 129S6/SvEv and C57BL/6, are able to alter the phenotypic expression of pituitary and pancreatic neuroendocrine tumours due to *Men1* mutations.

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OC5.2

The role of microRNA miR-34a in the regulation of aryl hydrocarbon receptor interacting protein

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Germline mutations in the aryl hydrocarbon receptor interacting protein (*AIP*) gene predispose to early onset pituitary adenoma, with a preponderance of somatotrophinomas. Patients harbouring an *AIP* mutation respond poorly to

somatostatin analogue (SSA) treatment. On the other hand, a subset of sporadic somatotrophinomas with no *AIP* mutations show low AIP protein expression and exhibit a decreased response to SSA treatment as well. microRNAs are small interfering RNAs which can regulate protein expression. miR-34a is predicted to interact with specific target sequences located within the 3′ untranslated region (3′UTR) of *AIP*.

RNA expression levels of *AIP* and miR-34a were evaluated in 31 sporadic somatotrophinoma tissues (17 invasive, 14 non-invasive) by RT-qPCR and immunohistochemistry was used to assess AIP protein expression. A luciferase reporter assay was used to examine the *in silico* predicted target sites of miR-34a in the 3′UTR of AIP. Deletion constructs of *AIP* 3′UTR were utilised to confirm the binding and regulatory function of miR-34a.

Low AIP protein expression was seen in invasive sporadic somatotrophinomas, whereas the corresponding mRNA levels were not statistically different compared to non-invasive tumours. This suggests that the expression of *AIP* might be regulated post-transcriptionally by miRNAs, which repress gene expression mainly by inhibiting protein translation. We observed that the expression levels of miR-34a is higher in somototrophinomas with low AIP expression. ($P=0.02$) Co-transfection of reporter cells with a miR-34a precursor and the luciferase-WT-AIP-3′UTR plasmid construct shows a negative effect on the luciferase expression, suggesting that miR-34a binds to the AIP-3″UTR. By using deletion mutants we have validated miR-34a predicted target sites at AIP-3″UTR.

Conclusions

We have identified and proved that miR-34a is a negative regulator of AIP protein expression and increased miR-34a expression could explain the low AIP expression and resulting tumour invasiveness and decreased SSA-responsiveness of sporadic somatotrophinomas.

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OC5.3

Whole-exome sequencing studies of non-functioning pituitary adenomas

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Pituitary non-functioning adenomas (NFAs), arising mostly from gonadotroph cells, represent the second most common type of pituitary tumour, after prolactinomas. NFAs are monoclonal in origin, but mutations of genes associated with hereditary pituitary syndromes (e.g. MEN1, AIP, CDKN1B, and PRKAR1A), classic oncogenes or tumour suppressor genes are rarely found. We therefore performed whole-exome sequence analysis to determine the tumourigenic events in pituitary NFAs using DNA extracted from seven pituitary NFAs and matched leucocyte samples. Informed consent was obtained from individuals using protocols approved by local and national ethics committees. The seven patients (four males, three females) had a mean age of 55 years (range 39–82 years). Histologically, all tumours were confirmed as pituitary gonadotroph adenomas with no atypia and a Ki-67 index of $\leq 3\%$. Exome capture was performed using the SureSelect Human All Exon 50Mb Kit and sequencing undertaken using the Illumina HiSeq2000 platform. Somatic variants were identified and validated. A high degree of coverage was achieved such that $\sim 97\%$ of targeted bases were represented by >10 bp reads. Twenty-four somatic variants were identified in the seven NFAs (mean $=3.5$ variants/tumour; range 1–7). The majority of variants occurred as missense single nucleotide variants (80%) with the remainder constituting synonymous changes or small frameshifting deletions. Each of the 24 mutations occurred in different genes, none of which had been previously reported to be associated with pituitary tumourigenesis. Three of the somatic mutations, occurring in independent tumours, represented putative driver genes involved in proliferation, apoptosis, and the cell-cycle check-point. However, analysis of these 3 genes in a validation set of 24 pituitary NFAs did not identify additional mutations. Thus, pituitary NFAs harbour few somatic mutations, consistent with their low proliferation rates and benign nature, but there appears to be no frequently mutated gene involved in the aetiology of pituitary NFAs.

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OC5.4

Clinical, metabolic, biochemical and radiological characterisation of patients with thyrotropinomas reveals a highly variable phenotype

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Background

Thyrotropinomas (TSHomas) are traditionally considered a rare albeit important cause of thyrotoxicosis, accounting for $\sim 1\%$ of all pituitary adenomas. Although early case series reported a preponderance of macroadenomas, emerging evidence suggests microadenomas are being increasingly diagnosed. In addition, the clinical/biochemical phenotype appears to be more variable than previously suspected. We therefore examined the clinical, metabolic, biochemical and radiological features of patients referred to our centre with a diagnosis of TSHoma over a 24-month period.

Methods

20 patients with hyperthyroxinaemia and non-suppressed TSH were identified, in whom laboratory assay artefact, confounding intercurrent illness/drug therapy and THRB mutations were excluded. Further investigations included: hyperthyroid symptom score, measurement of resting energy expenditure (REE), sleeping heart rate (SHR), bone mineral density (BMD), sex hormone-binding globulin (SHBG), α-subunit (ASU):TSH molar ratio, TRH test, OGTT, octreotide suppression test and volume MRI. 14 patients proceeded to a formal trial of somatostatin receptor ligand (SRL) therapy.

Results

Clinical/metabolic features varied markedly, ranging from euthyroid to overtly hyperthyroid, and were not clearly correlated with the degree of hyperthyroxinaemia; $\sim 70\%$ had evidence of cardiac (arrhythmias) and/or bone (osteopenia/osteoporosis) complications. Basal TSH levels were normal in 14 (70%) patients, and most exhibited a blunted response to TRH stimulation, but the fold-rise varied from 1.1 to 8. In one third of patients, SHBG levels were not raised (associated with evidence of co-existing GH hypersecretion in two (33%) cases). Similarly, the ASU:TSH molar ratio was not uniformly elevated. In $\sim 40\%$ of cases, volume MRI revealed a microadenoma; no demonstrable lesion was seen in two patients. SRL therapy normalised TFTs in 85% of patients, typically within 1 week of starting treatment. To date, eight patients have proceeded to surgery with histological confirmation of the diagnosis of thyrotropinoma.

Conclusion

The clinical, biochemical and radiological phenotype of thyrotropinomas is highly variable, with many cases exhibiting one or more atypical features often leading to diagnostic confusion.

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OC5.5

Densely and sparsely granulated somatotroph adenomas: clinical, genetic and histological differences

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Somatotroph adenomas causing acromegaly are histologically classified into densely and sparsely granulated subtypes and an intermediate, mixed type. Although the different subtypes are not currently taken into account when making decisions about the management of acromegaly, there is growing evidence that the subtypes represent clinically different entities.

In a cohort ($n=52$) of somatostatin-naïve patients with acromegaly, sparsely granulated adenomas were larger ($P=0.038$), found predominantly in younger ($P=0.029$), female patients ($P=0.026$) and exhibited higher proliferation indices and invasion of surrounding structures ($P<0.0001$ and $P=0.001$). Sparsely granulated adenomas also showed diminished responses to the octreotide suppression test compared to the densely granulated subtype ($P=0.007$). Codons

201 and 227 of Gsα and codon 49 of GHR were sequenced and the mutation status compared to clinical characteristics. There were no mutations at codon 49 of GHR in this cohort and mutation in Gsα did not co-segregate with granulation pattern. However, Gsα mutation was associated with smaller tumours ($P=0.027$) with a greater production of growth hormone ($P=0.048$) and more common satisfactory response to the octreotide suppression test ($P=0.022$). Immunohistochemical characterisation of the adherens junction complex in a subset of this cohort revealed an intact complex at the cell membranes of densely granulated adenomas that was disrupted in the sparsely granulated subtype. Disruption of cell–cell adhesion may underlie the poorly cohesive and more invasive features of sparsely granulated adenomas.

The granulation pattern of somatotroph adenomas is associated with differing clinical, histological and biochemical characteristics in this cohort. The sparsely granulated subtype represents a larger, more invasive tumour with disrupted cell–cell adhesion. Determination of the adenoma subtype may become an important consideration in the management of acromegaly.

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OC5.6

Manipulating PBF/PTTG1IP phosphorylation status to improve radioiodine uptake in thyroid and other tumours

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The clinical effectiveness of ablative radioiodine treatment is limited by the ability of the sodium iodide symporter (NIS) to uptake ^{131}I. A significant proportion of well-differentiated thyroid tumours are unable to concentrate sufficient radioiodine for effective therapy, and in other tumour models such as breast, where radioiodine uptake would be an attractive therapeutic option, uptake is insufficient. Pituitary tumor-transforming gene-binding factor (PBF/ PTTG1IP) is over-expressed in multiple cancers including thyroid and breast, and potently represses NIS function in thyroid cancer. We now demonstrate that the post-translational mechanism by which PBF represses NIS is applicable to non-thyroid tumour cells including breast and prostate. Crucially, we describe a method by which PBF repression of NIS may be overcome in human tumours. We identify PBF as a tyrosine phospho-protein which specifically binds the proto-oncogene tyrosine-protein kinase Src in mass spectrometry, GST-pulldown and co-immunoprecipitation assays. Src induction leads to phosphorylation at PBF residue Y174. Abrogation of this residue results in plasma membrane retention and an inability to bind NIS. The Src inhibitor PP1 inhibits PBF phosphorylation in multiple cell lines *in vitro*, including human primary thyroid cells. Of direct clinical importance, PP1 overcomes PBF repression of iodide uptake in human primary thyroid cells. Thus, we propose that targeting PBF phosphorylation at residue Y174 via tyrosine kinase inhibitors may be a novel therapeutic strategy to enhance the efficacy of ablative radioiodine treatment in thyroid and other endocrine and endocrine-related tumours.

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OC5.7

Uterine tumours with loss of progesterone receptor expression develop in mice deleted for a cell division cycle 73 allele

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Mutations of the cell division cycle 73 (CDC73) gene, which encodes the 531 amino acid protein parafibromin, are associated with the Hyperparathyroidism-Jaw Tumour (HPT-JT) syndrome, an autosomal dominant disorder characterised by parathyroid tumours and ossifying jaw fibromas. In addition, ~75% of women with HPT-JT develop benign and malignant uterine tumours, which include endometrial hyperplasia, adenosarcomas, adenofibromas, and leiomyomas. To explore the role of *CDC73* in uterine tumourigenesis, we developed a mouse model deleted for one *Cdc73* allele ($Cdc73^{+/-}$). Mice were kept in accordance with welfare guidelines and project licence restrictions. Female $Cdc73^{+/-}$ and wild-type ($Cdc73^{+/+}$) mice were studied at ≧18 months of age, and proliferation was assessed by administration of five-bromo-two-deoxyuridine in drinking water for two weeks. Uterine tumours, which included adenofibromas and adenomyomas developed in 33% of $Cdc73^{+/-}$ mice (8 of 24 mice), but in none of 23 wild-type littermates. These $Cdc73^{+/-}$ mice also had several other endometrial histological abnormalities which included: large wall cysts; hyperplasia with squamous metaplasia; and transluminal bridging. Furthermore, immunohistochemistry demonstrated reduced parafibromin expression and loss of progesterone receptor expression in the uterine hyperplasia and tumours of these $Cdc73^{+/-}$ mice, when compared to that in normal uteri from $Cdc73^{+/+}$ mice. In addition, proliferation rates in uterine myometria from $Cdc73^{+/-}$ mice were significantly increased compared to myometria from $Cdc73^{+/+}$ littermates ($0.900\pm0.168\%$ vs $0.526\pm0.063\%$, respectively, $P<0.05$). Thus, one third of female $Cdc73^{+/-}$ mice developed uterine hyperplasia and tumours with reduced parafibromin expression, loss of progesterone receptor expression, and increased cellular proliferation rates. These female $Cdc73^{+/-}$ mice may therefore provide a useful model for the study of aetiological molecular mechanisms and treatments for uterine tumours.

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OC5.8

Pituitary adenoma and phaeochromocytoma/paraganglioma – a novel syndrome with a heterogeneous genetic background

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Pituitary adenomas and phaeochromocytoma/paragangliomas (PHAEO/PGL) can very rarely occur in the same patient or in the same family. Together, they are not known to be part of any classical endocrine neoplasia syndromes. In some caes the pathogenetic mechanism may be secondary to a PHAEO secreting GHRH leading to somatotroph hyperplasia and clinical acromegaly. However, we suggest several other mechanisms which could lead to the development of pituitary and PHAEO/PGL together: a known PHAEO/PGL gene which also causes pituitary adenoma formation, a known pituitary tumour gene which also causes PHAEO/PGL, digenic disease, a new gene(s) causing both diseases, and one must not exclude the possibility that the development of the two tumours together might be coincidence. We found 52 cases in the literature with this combination of diseases, although only 15 of them had a confirmed diagnosis.

We studied 25 patients with the combination of pituitary adenoma and a PHAEO/PGL. Recognised PHAEO/PGL causing genes (*SDH A-D, SDHAF2, RET, VHL, TMEM127, MAX*) and pituitary adenoma genes (*MEN1, AIP, CDKN1B*) were sequenced using next generation or Sanger sequencing, and loss of heterozygosity was studied in the tumours, where available.

We identified mutations in *SDHB, SDHC, SDHD, MEN1, RET* and *VHL* in some patients and families with PHAEO/PGL and pituitary adenomas (1 *SDHA* variant of unknown significance, 1 *MEN1* variant with unknown pathogenicity and several mutations; 4 *SDHB*, 1 *VHL*, 1 *SDHC*, 1 *SDHD* and 1 *MEN1*). Loss of heterozygosity for the relevant gene was shown in all the cases where pituitary tissue was available. In addition, we noted that pituitary adenomas in patients affected by *SDH* mutations have unique histology.

These data suggest that mutations in some PHAEO/PGL and pituitary genes can affect both these tissue types.

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Poster Presentations

Bone

P1

GNA11 loss-of-function mutations cause familial hypocalciuric hypercalcaemia type 2 (FHH2)

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Loss-of-function mutations of the calcium-sensing receptor (CaSR), a G-protein-coupled receptor (GPCR), result in familial hypocalciuric hypercalcaemia (FHH), a disorder of extracellular calcium homeostasis affecting the parathyroids and kidneys. However, around 35% of FHH patients do not have CaSR mutations. A form of FHH, designated FHH2, has been mapped to chromosome 19p. The *GNA11* gene, encoding G-protein α11 ($G\alpha_{11}$), a component of the CaSR signalling pathway, resides on 19p and is therefore a candidate gene for FHH2. We investigated the FHH2 kindred for *GNA11* mutations and identified an in-frame isoleucine deletion (Ile199del). *GNA11* mutational analysis was also undertaken in nine previously reported FHH patients who did not have CaSR mutations, and this revealed a Leu135Gln missense mutation in one of these patients. To assess the functional consequences of these mutations, wild-type and mutant $G\alpha_{11}$ proteins were expressed in HEK293 cells stably transfected with CaSR, and the intracellular calcium response to changes in extracellular calcium was measured. The Ile199del and Leu135Gln mutations both led to a rightward shift of the concentration-response curves with significantly ($P<0.0001$) increased mean EC50 values of 2.52 mM (95% confidence interval (CI) = 2.49–2.56) and 3.46 mM (95%CI = 3.40–3.51), respectively, when compared to the wild-type $G\alpha_{11}$ EC50 of 2.29 mM (95%CI = 2.24–2.34). These findings indicated that the Ile199del and Leu135Gln mutations were associated with a loss of $G\alpha_{11}$ function. An examination of the crystal structure of $G\alpha_q$, which has 90% amino acid identity to $G\alpha_{11}$, indicated that both of these mutations were located in Gα structural motifs that are critical for GPCR signalling. In particular, the Ile199del mutation disrupted hydrogen bonding within a peptide loop that comprises part of the Gα-GPCR interface. This study has identified the genetic abnormality causing FHH2, and further increased our understanding of extracellular calcium homeostasis and the structure–function relationships of G-protein α subunits.

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P2

Increased linear bone growth in SOCS2 knockout mice in response to GH is independent of systemic or local IGF-1

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Introduction
GH signalling is essential for post-natal linear bone growth. The systemic/local mechanisms responsible for GH action remain unclear as the importance of liver derived IGF1 on linear growth has recently been challenged.
Aim
To unravel the underlying mechanisms of linear bone growth we exploited the suppressor of cytokine signalling-2 (SOCS2) KO mice which have enhanced linear growth despite normal systemic IGF1 and GH levels.
Methods
Growth plates were micro-dissected from WT and SOCS2 KO bone and IGF1 levels assessed by RT-qPCR. Embryonic day 17 metatarsals were cultured from both WT and SOCS2 KO mice in the presence of GH in order to assess downstream signalling and IGF1 expression.
Results
Our present *in vivo* data revealed no downstream increase in *Igf1* expression in growth cartilage of WT and SOCS2 KO mice. These data were extended by *ex-vivo* embryonic metatarsal experiments. In response to GH, wild-type (WT) bones expressed increased SOCS2 (but not SOCS1 or 3) transcript levels but STAT5 phosphorylation was profoundly less than that noted in similarly treated SOCS2 null metatarsals. This confirms and extends our previous *in vitro* chondrocyte data. Increased STAT5 activation of SOCS2 metatarsals following GH challenge was associated with increased linear growth over an 8-day-period whereas the growth of GH treated WT bones remained unchanged. This increased growth of SOCS2 null bones in response to GH was not, however, accompanied by greater IGF1 and IGFBP3 transcript levels suggestive of IGF1 independent mechanisms. Moreover, GH remained stimulatory to SOCS2 bone growth in the presence of an IGF1 receptor inhibitor (NVP-AEW541).
Discussion
These studies emphasise the importance of SOCS2 in the regulation of GH stimulation of linear bone growth and indicate that GH can enhance linear growth by initiating molecular pathways intrinsic to the growth plate that are independent of local IGF1 production.

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P3

Mutations in CLC-5 cause disturbances in cytoskeletal dynamics and solute transport in Dent's disease renal proximal tubule cell-lines

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Dent's disease is a renal proximal tubular Fanconi disorder characterised by generalised loss of solutes incuding insulin, glucose, PTH, amino acids and vitamin-D binding protein and is associated with rickets in 25% and phosphaturia in ~40% of patients. Dent's disease is caused by mutations in the chloride/proton antiporter CLC-5, which, with megalin and cubilin has a role in receptor-mediated endocytosis and vesicle trafficking. To further elucidate the role of CLC-5 in endosomal trafficking, we performed gene expression profiling using Illumina's Human-HT2v4 BeadChip utilising three human conditionally-immortalised proximal tubular epithelial cell-lines (ciPTECs) that harboured one of three CLC-5 mutations: an in-frame histidine insertion at codon 30 (30:insHis), a deletion of codons 132 to 241 (del132–241) and a nonsense mutation (Arg637Stop) ($n=5$ for each ciPTEC). Differentially expressed genes with a minimal fold-change of 1.5 and a $P<0.05$ were further analysed. Sixty-seven genes were differentially expressed in all three Dent's disease ciPTECs, and individually, the ciPTECs had 107 (30:insHis), 272 (del132-241) and 375 (Arg637Stop) differentially expressed genes. Pathway analysis revealed that two functional pathways, which involved endocytosis/actin dynamics and solute transport, were commonly affected. Quantitative PCR and Western blot analysis confirmed that promoters of actin polymerization were upregulated and inhibitors of actin polymerisation were downregulated in Dent's disease ciPTECs; these would lead to increased plasma membrane directed vesicular movement, and reduced trafficking from membrane to early endosome. In addition, several small GTPases that regulate early endosomal trafficking were downregulated. Furthermore, Dent's disease ciPTECs had decreased expression of a number of solute transport genes including *SLC38A5*, an amino acid transporter, and glucose transporters, which may contribute to the observed aminoaciduria and glycosuria in patients. Investigation of these dysregulated pathways may help to elucidate the mechanisms by which CLC-5 mutations cause Dent's disease.

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P4

Contribution to bone mass and strength of osteoblast GH actions that are independent of local IGF-1 production: lessons from the SOCS2 knockout mouse

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GH is anabolic to the skeleton but its mode of action is unclear. Clues are available from the study of the suppressor of cytokine signalling-2 (SOCS2) KO mouse which has increased bone mass despite normal systemic IGF1 levels. Whilst suggesting direct GH effects on bone forming osteoblasts the precise signalling mechanisms remain unclear. The aims of this study were, therefore, to further detail the bone phenotype of SOCS2 KO mice and determine if GH promotes bone mass by mechanisms that are dependent or independent of osteoblast IGF1 production.

All SOCS2 KO mice had increased trabecular BV/TV ($P<0.05$) and cortical BV ($P<0.05$) as assessed by μCT. The cortex was thicker only in male SOCS2 KO mice and this was associated with increased breaking strength ($P<0.05$) as determined by three-point bending. IGF1 and IGFBP3 mRNA expression levels were similar between bone and liver samples of SOCS2 KO and WT mice supporting a local, IGF1 independent, GH mechanism responsible for the noted increased bone mass. *In vitro* mechanistic studies (immunoblotting and immunocytochemistry) indicated that in WT osteoblasts, SOCS2 (but not SOCS1 or 3) transcript and protein expression increased in response to GH. Also, GH promoted greater STATs one, three and five phosphorylation in SOCS2 KO calvarial osteoblasts than in WT cells. Overexpression of SOCS2 in MC3T3 osteoblast-like cells resulted in decreased STAT 5 signalling following GH challenge. This GH mediated increased STAT signalling in SOCS2 KO cells resulted in, as expected, elevated IGF1 mRNA expression ($P<0.05$) but the magnitude of the increase was similar to that noted in WT cells. These data confirm our *in vivo* observations.

These studies emphasise the critical role for SOCS2 in controlling GH anabolic bone effects. They also confirm the functional contribution to bone mass of osteoblast GH actions that are independent of local IGF1 production.

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P5

Mimicking osteocytes *in vivo* using 3D collagen gels: development of a novel tool to study osteocyte biology

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Osteocytes make up >90% of bone cells, are embedded in mineralised matrix where they form a communication network. Osteocytes differentiate from osteoblasts, and are thought to be mechano-sensitive. They are very difficult to isolate leading to a dependence on cell lines for *in vitro* studies of osteocyte biology. There is thus a need to develop new methods to study these cells. Recent publications indicate that osteoblasts maintained in *in vitro* 3D collagen gels may differentiate to osteocytes.

We maintained osteoblasts (MC-3T3; human primary) in 3D type I collagen gels (250 μl; 48-well plates) for 15 days in either α-MEM (basal medium), or mineralising medium (basal medium, dexamethasone, β-glycerophosphate). Cell number, viability and phenotype (IHC, qRTPCR, confocal microscopy), gel stiffness (Losenhausen), and VEGF and IL6 secretion (ELISA) were quantified. Cells appeared more dendritic over time and formed connecting cellular networks (H&E, phalloidin). Cell viability was similar in both media (>85% MC-3T3s; >95% human primary), but cell numbers were significantly higher ($P<0.001$) in mineralising conditions. Calcein and alizarin red staining confirmed the presence of mineralisation from day 7. DMP-1 (osteoblast/osteocyte differentiation marker) was not expressed (IHC) at day 3 but then gradually increased in expression (days 7–14). E11 (osteocyte marker localised to dendrites) was low at day 3 (IHC, qRTPCR), peaked at day 10, and returned to lower levels by day 14. Gel stiffness significantly increased over 11 days ($P<0.01$) and the mineralised gels were stiffer than those in basal medium ($P<0.01$). VEGF and IL6 secretion also changed significantly with time and culture conditions.

Osteoblasts maintained in 3D gels appear to differentiate along the osteocytic pathway. It is possible to mineralise these cultures thus mimicking further their *in vivo* environment. This methodology provides a novel model to study osteocyte biology, and will enable studies relating to bone loading, repair and regeneration.

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P6

Retinoic acid and IGF-1 stimulate the differentiation of human primary osteoblasts to osteocytes in 3D collagen gels

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Osteocytes differentiate from osteoblasts, are embedded in mineralised matrix and are critical regulators of bone remodelling. *In vitro* osteocyte models are currently limited to cell lines in monolayer, but these do not represent their 3D environment *in vivo*. We have recently shown that osteoblasts in 3D gels differentiate along the osteocytic pathway. Since retinoic acid (RA) has been shown to stimulate monolayer osteoblast/osteocyte differentiation, we have investigated the effects of RA on cells in 3D. We have also hypothesised that IGF1 modulates osteocyte differentiation and function.

We maintained osteoblasts in 3D type I collagen gels (up to 15 days) +/− RA (5×10^{-6}M) or IGF1 (5 μg/ml). We measured cell number and viability (trypan blue), VEGF and IL6 secretion (ELISA), and expression and secretion of osteocyte related proteins (e.g. DMP1, RANKL, FGF-23; qRTPCR, ELISA).

RA significantly ($P<0.001$) reduced cell numbers (HOBS, MC-3T3s), whereas IGF1 had no effect on either cell type. Cell viability was high throughout. In HOBS, IL6 secretion was decreased at day 7 ($P<0.001$), whereas IGF1 had no effect. In MC-3T3s both compounds decreased IL6 secretion. Interestingly, both RA and IGF1 significantly increased RANKL expression and stimulated FGF-23 expression and secretion in MC3T3 cells, confirming that the cells had differentiated to osteocytes. Both compounds also increased VEGF secretion in these cells.

RA and IGF1 modulate mouse and human osteoblast/osteocyte number and function in 3D gels, with broadly similar results obtained with the two cell types tested. The production of FGF-23 in the presence of IGF1 highlights the possible role of IGF1 in osteocyte differentiation and function. Since we have also recently developed a method of applying load to these gels, this study provides a novel 3D *in vitro* system to further study the role of IGF1 in osteocyte differentiation and function, especially those related to mechano-sensing signalling pathways.

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P7

Alterations of CLC-5 expression, function and trafficking in Dent's disease

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Dent's disease, due to mutations in the chloride/proton antiporter, CLC-5, represents one form of familial hypophosphataemic rickets. Dent's disease patients also have: low-molecular-weight-proteinuria; hypercalciuria with nephrolithiasis and renal failure; and urinary loss of parathyroid hormone and vitamin D-binding protein, due to defective receptor-mediated endocytosis within the renal proximal tubule. However, there is variability in these clinical phenotypes such that only 25% of patients have rickets, while ~40% have phosphaturia, and we have previously demonstrated that this is also affected in the cellular phenotypes of human conditionally-immortalised proximal tubular epithelial cell-lines (ciPTECs) established from the urines of patients harbouring CLC-5 mutations. To further elucidate the mechanisms underlying the differences in the cell phenotypes and their structural-functional relationships we studied two ciPTECs harbouring CLC-5 mutations in different intracellular domains: an in-frame insertion (30:insHis) in the N-terminus that lies in a highly charged region; and a nonsense mutation (Arg637Stop) in the C-terminus, that causes loss of a region of CLC-5 known to interact with endocytic accessory proteins. Heterologous expression and whole-cell patch-clamp recordings within HEK293

cells revealed CLC-5 mutations to cause a reduction in chloride currents, to ~70% of wild-type for 30:insHis-CLC-5 ($n=10$), and ~30% of wild-type for Arg637Stop-CLC-5 ($n=6$) ($P<0.05$ in both). Confocal microscopy of ciPTECs demonstrated that 30:insHis-CLC-5 had reduced cell surface expression, while the Arg637Stop-CLC-5 was predominantly intracellular. Thus, the Arg637Stop-CLC-5 mutation may disturb interactions with endocytic proteins, thereby disrupting the trafficking of CLC-5 to the cell surface, and reducing the overall chloride conductance of the cell; whilst the 30:insHis-CLC-5 mutation which alters the amino acid charge balance of the N-terminus, likely affects chloride conductance, thereby impairing endosomal acidification and receptor-mediated endocytosis. These studies further demonstrate that different intracellular mechanisms give rise to Dent's disease and these may account for the differences in patient phenotypes.

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P8

Excessive GH expression in bGH transgenic mice adversely alters bone architecture and quality

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GH is an important anabolic hormone involved in the regulation of longitudinal bone growth. However, acromegaly patients have a higher prevalence of vertebral fractures despite normal bone mineral density (BMD), suggesting that over-expression of GH has adverse effects on skeletal architecture and strength. We used giant bovine GH (bGH) transgenic mice to analyse the effects of high serum GH levels on bone architecture and mechanical strength. Five month-old hemizygous male bGH mice were compared with age- and sex-matched wild type (WT) controls ($n=7$ in each group). Tibia and lumbar vertebrae were harvested from these mice and BMD and bone architecture analysed using micro-computed tomography. Femora were tested to failure using three-point-bending. As expected, bGH transgenic mice displayed significant increases in body weight and lengths of tibiae and vertebrae. Both cortical and trabecular bone compartments were altered in bGH tibia compared to WT ones. bGH mice showed decreases in trabecular bone volume fraction (BV/TV) (−49%) and trabecular number (−48%), while trabecular pattern factor (+797%) and structure model index (+68.9%) were significantly increased indicating deterioration in trabecular bone structure and connectivity. Although cortical tissue perimeter was drastically increased in transgenic mice (+53.2%), cortical thickness was reduced by 25%. bGH mice showed similar trabecular BMD in lumbar vertebra (L4) relative to controls, while cortical BMD was significantly reduced in bGH vertebra compared to controls. Mechanical testing of femora confirmed that bGH femora have decreased intrinsic mechanical properties compared to WT, including ultimate stress (−27.6%) and Young's modulus (−54.1%). Preliminary histomorphometry results also indicate that bone resorption is increased in bGH tibia compared to controls. These data collectively suggest that high serum GH levels negatively affects bone architecture and quality and that bGH transgenic mice are a useful model to understand the mechanisms involved in the skeletal changes observed in acromegaly patients.

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P9

Bone health in type 1 diabetes patients with celiac disease

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Objectives
Type 1 diabetes mellitus (T1DM) is associated with various autoimmune conditions including celiac disease. Both these conditions are independently and variably associated with risk of osteoporosis. The current study intended to study bone health parameters and factors affecting it in patients with T1DM with serological evidence of celiac disease (CD).
Methods
A cross sectional study including 100 type one diabetes patients following up in our hospital was screened for CD bye IgA tissue transglutaminase (TTG) levels. Twelve patients (12%) patients tested positive. Twenty age and sex matched T1DM (IgA TTG negative) patients served as controls. After history and physical examination, biochemical parameters including serum levels of ionized calcium, inorganic phosphorus, alkaline phosphatase, parathyroid hormone and 25 hydroxy vitamin D were measured. Bone mineral density (BMD) were measured at total body (TB), lumbar spine (LS) and left femoral neck (FN) using dual energy X ray absorptiometry (Lunar DRX DPO). Similarly DXA scan was done for measurement of total body bone mineral content (TBBMC), bone area (TBBA) and body composition. All the parameters were expressed as mean ± S.D. Data were analyzed using online graphpad quickcalc software and $P<0.05$ was considered statistically significant.
Results
TBBMD (0.77 ± 0.04 vs 0.81 ± 0.05 g/cm^2) and TBBMC (801 ± 143 vs 982 ± 196) were lower in type one diabetic subjects with IgA TTG positivity ($P<0.05$). Similarly the total body Z score (−1.64 ± 0.56 vs −0.46 ± 0.67), lumbar spine Z score (−1.42 ± 0.61 vs −0.22 ± 0.83) and femoral neck Z score (−1.48 ± 0.52 vs −0.34 ± 0.79) and TBBMC for age Z score (−1.3 ± 0.8 vs −1.0 ± 0.9) were lower in type 1 diabetic subjects with IgA TTG positivity ($P<0.05$). However, TBBA (1038 ± 149 vs 1134 ± 156 cm^2) and TBBA for age Z score (−0.9 ± 0.9 vs −0.8 ± 0.9) did not significantly differ between the two groups.
Discussion
Celiac autoimmunity is associated with reduced bone mineralization in T1DM patients. Celiac disease should be considered as a possible secondary cause of osteopenia in type 1 diabetic patients found to have a reduced BMD.
Conclusion
Important impact of early identification of CD in T1DM could be to prevent this important complication.

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P10

High throughput detection of early joint pathology in mouse models of osteoarthritis

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Articular cartilage maintenance and repair is regulated by numerous endocrine and paracrine factors. Investigation of molecular mechanisms underlying osteoarthritis (OA) is limited by inability to identify early stage disease and individuals at risk of progression. Susceptibility to OA is genetically determined and the availability of mice from the International Knockout Mouse Consortium with deletions of every known gene provides a unique opportunity to investigate its pathophysiology. Nevertheless, screening for joint abnormalities using standardized Osteoarthritis Research Society International (OARSI) histology protocols is labour intensive and costly, thus limiting progress. We hypothesize that the triiodide ion in Lugol's iodine solution penetrates the intact joint to provide excellent contrast resolution and permit simultaneous imaging of unmineralized and mineralized tissues. The aim of this study was to investigate triiodide staining in knee and hip joints from WT mice to determine morphological and structural parameters of articular cartilage and subchondral bone.
Limbs from WT mice were fixed and stored in isotonic 70% ethanol and images of knee and hip joints obtained using digital x-ray microradiography. To determine the optimal osmolality and triiodide concentration, samples were placed in varying dilutions of Lugol's iodine solution. To investigate the optimal duration of staining and X-ray intensity required, samples were imaged between 24 and 144 h using accelerating voltages of 16–35 kV. Staining for 48 h in a 25% saturated solution of Lugol's iodine (308 mOsmol/l) achieved excellent soft tissue contrast at 31 kV and 19 s exposure and resulted in no demonstrable tissue shrinkage.
These studies demonstrated that triiodide staining results in excellent visualization of the joint capsule, menisci, articular cartilage and mineralized tissue establishing that this method can be used for high resolution three-dimensional imaging using micro-CT and electron microscopy.
This strategy provides a novel approach for high throughput detection of early joint disease in genetically modified mice.

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P11

Bone health in children with GH deficiency

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Objectives
The current study intended to assess the impact of GH deficiency (GHD) on bone health after using various size corrections.
Methods
Thirty prepubescent children with GHD (male:female = 20:10, mean age– 9.4 ± 3.5 years) were included in the study. Data on anthropometry and total body bone mineral content (TBBMC), bone area (TBBA) and lean body mass (TBLBM) by dual energy X ray absorptiometry were collected. Anthropometric *Z* scores and bone parameter *Z* scores were computed using ethnic normative reference database.
Results
Mean height for age *Z* score (HAZ) was −5.1 ± 1.7. Mean TBBMC for age *Z* score was −9.2 ± 6.3 and mean TBBA for age *Z* score was −7.1 ± 4.3. All the study children had 'short bones' with HAZ < −2. Twenty-four (80%) children had 'narrow bones' (TBBA for height *Z* score < −2). Twenty one (70%) children had 'light bones' (TBBMC for TBBA *Z* score < −2). Mean TBBMC for age *Z* scores were significantly lower than the mean HAZ ($P < 0.05$), indicating lower BMC after adjusting for height. Mean TBBMC for TBLBM *Z* score was – 3.3 ± 4.2, indicating bone mineral deficit even after adjusting for TBLBM. There was no significant gender difference in any of the parameters.
Discussion
GHD in children causes low bone mineral density (BMD). Height and muscle force drive bone mineralization. International society of clinical densitiometry has made it obligatory to applying size corrections. Analysis of different bone health parameters lead to the demonstration that Indian children with GHD have 'short bones' (100% cases), 'narrow bones' (80% cases) and 'light bones' (70% cases).
Conclusion
Indian prepubertal GHD children had low bone mass even after applying size corrections implying need for corrective measures for their bone health.

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P12

Vitamin D receptor polymorphisms and bone mass indices in post menarcheal Indian adolescent girls

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Objective
The aim of the present study was to assess the association between vitamin D receptor (VDR) gene polymorphism and bone mass indices in Indian adolescent girls.
Methods
The current study was a cross sectional one including 100 post menarcheal girls aged 15–18 years. Serum levels of ionized calcium, inorganic phosphorus, alkaline phosphatase, parathyroid hormone and 25 hydroxy vitamin D were measured. bone mineral content (BMC), bone area (BA) and bone mineral density (BMD) were measured at total body (TB), lumbar spine (LS) and left femoral neck (FN) using dual energy X ray absorptiometry (Lunar DRX DPO). Polymorphisms of VDR gene at the Fok1 and Bsm 1 loci were detected using SYBR green quantitative PCR.
Results
Vitamin D deficiency (serum 25-OH D3 < 30 ng/ml) was observed in 43% patients. The overall prevalence of genotype for Bsm1 in this study was 33.3% Bb, 29.2% bb and 37.5% BB. For Fok1 genotype, the prevalence was 44.2% Ff, 7.5% ff and 48.3% FF. There were no significant differences in the blood parameters when classified according to Bsm1 and Fok1 genotypes. Subjects with BB genotype have significantly higher mean TBBMC (1012 ± 178 g), TBBA (1264 ± 186 cm^2), TBBMD (0.89 ± 0.06 g/cm^2) and LSBMD (0.81 ± 0.04 g/cm^2) than Bb and bb ($P < 0.05$). They showed tendency for association with LSBMC and LSBA ($P < 0.1$). Bsm 1 genotype did not show an association with FN bone indices whereas Fok1 genotype did not show an association with TB, LS or FN bone indices.
Discussion
Vitamin D is important for bone health. Vitamin D deficiency is common among children and adolescents in India, in spite of abundant sunshine. With respect to the Bsm1 genotype, the Bb and bb subgroups were more prevalent (62.5%) than BB (37.5%) and were associated with worse bone health parameters. Whereas with respect to the Fok1 polymorphism, FF genotype was most common (48.3%). But there was no difference in the bone health parameters among different subgroups.
Conclusion
The present study demonstrates VDR gene polymorphism; defined by Bsm 1 genotype has an influence on total body and lumbar spine bone mass indices in post menarcheal Indian girls.

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P13

Identification of a novel heterozygous mutation in exon 50 of the COL1A1 gene manifesting clinically as osteogenesis imperfecta

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Introduction
Osteogenesis imperfecta (OI) is a rare, heterogeneous, genetic connective tissue disorder that manifests clinically as bone fragility, brittleness and growth disorder. Effective diagnosis is important (although often challenging) to enable institution of early and effective multidisciplinary management.
The case
A 19-year-old woman was referred to the Endocrine clinic at the Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism (WISDEM, UHCW) following a low-impact fall resulting in fractures of her pelvic rami. Her past medical history revealed numerous childhood low-impact fractures, a prior eating disorder and poor hearing. There was no family history of note. Her only medication was the combined oral contraceptive pill (microgynon), and she was a smoker. On examination, her BMI was 17.9 kg/m^2. She had blue discolouration to her sclerae bilaterally, bilateral clinodactyly and increased joint laxity. Weber's test localised to the right-side. Serum calcium, magnesium, phosphate, alkaline phosphatase and 25-hydroxycholecalciferol levels were all normal. Pelvic X-rays confirmed fractures to the superior and inferior rami. DEXA bone scan revealed lumbar osteopenia (*T*-score −1.8).
Genetics
Sequencing genomic DNA revealed that she is heterozygous for the c.3880_3883dup mutation in exon 50 of the *COL1A1* gene, confirming our clinical suspicion of OI Type 1. This mutation is predicted to result in a frameshift at p.Thr1295, and truncating stop codon 3 amino acids downstream. This mutation has not previously been reported in OI. She has ongoing genetics and audiology follow-up, has been commenced on a bisphosphonate and has been advised regarding modifications to her lifestyle.
Conclusions
We report a novel frameshift mutation within *COL1A1* resulting in OI in a young woman who had sustained numerous childhood low-trauma fractures. Despite her classical blue sclerae, the diagnosis of OI had not previously been entertained. This case illustrates the importance (and challenges) of early diagnosis and effective management of OI.

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P14

Bone mass accrual following supplementation of vitamin D alone versus vitamin D + calcium in underprivileged Indian premenarcheal girls

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Objective
To determine effectiveness of supplementing vitamin D alone vs vitamin D + calcium on bone mass accrual in underprivileged Indian premenarcheal girls.
Methods
A double blind, matched pair, cluster randomization study was carried out in 200 premenarcheal girls (8–12 years) from three public schools. The participants were

randomized into two clusters and were allocated to receive either vitamin D (Group A): 30 000 IU oral cholecalciferol every 3 months or vitamin D + calcium (Group B): 500 mg/day calcium and vitamin D 30 000 IU oral cholecalciferol every 3 months. The supplementation trial was done for the duration of 1 year. Anthropometry, biochemical parameters, total body bone area (TBBA), mineral content (TBBMC) and bone mineral density (TBBMD) by dual energy X ray absorptiometry were assessed at baseline and at the end of one year.
Results
At baseline vitamin D deficiency was observed in 84 (42%) girls. Post supplementation TBBMC, TBBMD and TBBA were significantly increased in both the groups in comparison to baseline. But the corresponding *Z* scores showed significant improvement only in group B. Mean percent increase in TBBMC was significantly higher in group B (from 841 ± 174 to 1018 ± 226 g, 22.3%) compared to group A (from 793 ± 138 to 935 ± 185 g, 17.6%, $P=0.02$). Improvement in TBBMC-for-age *Z* score was higher in the group B (from −1.1 ± 0.9 to −0.9 ± 0.9, 22%) vs group A (from −1.1 ± 0.7 to −1.1 ± 0.8, 13.6%, $P=0.03$). Similarly increments in TBBMD was significantly higher in group B (from 0.78 ± 0.05 to 0.82 ± 0.06 g/cm^2, 5.5%) vs group A (from 0.77 ± 0.05 to 0.80 ± 0.05 g/cm^2, 3.3%, $P=0.03$). However increase in TBBA was not significantly different between the two groups (14.4% in group B vs 13.8% in group A, $P>0.1$). No significant difference in mean percent increase in TBBMC were observed across vitamin D categories (<20, 20–30, >30 ng/ml) in both the groups. The increase in height was similar in the two supplemented groups (7.3 ± 1.5 cm in group A vs 7.4 ± 1.4 cm in group B).
Discussion
Low adult bone mass is linked to osteoporosis and fractures and is dependent on the extent of childhood and adolescent bone mineralization. Indices of bone health improved significantly following calcium and vitamin D supplementation.
Conclusion
Calcium along with vitamin D supplementation was more effective in improving bone mass accrual in underprivileged premenarcheal girls than vitamin D alone.

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P15

Primary hyperparathyroidism in patients with urolithiasis: prevalence and predictors

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Objective
To know the prevalence of primary hyperparathyroidism (PHPT) in urolithiasis and predictors of PHPT in urolithiasis.
Methods
This cross sectional study was conducted between July 2005 and July 2012 in Department of Endocrinology and Urology at our hospital. In this study a total of 165 consecutive patients with urolithiasis with radioopaque stones were evaluated for clinical and biochemical profile. Stones retrieved were analyzed for type of stone using infrared spectroscopy.
Results
Out of these 165 patients, 123 were males, 42 females, with a mean age of 34.5 ± 12.1 years. Eleven patients (7%) had histopathologically proven PHPT. Mean age of these patients was 45.6 ± 12.4 years with male:female ratio of 4:7. Prevalence of bone pains, backache, fracture, weakness, fatigability, joint pain, and myopathy were more common in PHPT group. Three (35%) patients in PHPT and 3 (2%) in non PHPT group had nephrocalcinosis ($P<0.0001$). Simultaneous renal and ureteric stones were significantly more common in PHPT patients ($P<0.0001$). Calcific pancreatitis was found in 2 (18.1%) patients with PHPT as compared to 1 (0.6%) in non PHPT group (*P* value- 0.0001).
Mean serum calcium in PHPT group was 12.3 ± 0.3 g/dl, 9.2 ± 0.03 g/dl in non PHPT group ($P<0.0001$), Alkaline phosphatase was 312.5 ± 73.9 vs 114.3 ± 2.6 IU/l ($P<0.0001$) and phosphate was 3.3 ± 0.3 vs 3.7 ± 0.5 g/dl (*P* value = 0.4), iPTH levels were 398.9 ± 132.4 vs 49.3 ± 3.1 pg/ml ($P<0.0001$) respectively. Calcium oxalates were the most common type of stones in either of the groups (85.3 vs 69.1%) respectively.
Discussion
The prevalence of PHPT in our patient with urolithiasis is 7%. Urolithiasis is more common in males but prevalence of PHPT is more common in females with urolithiasis. Urolithiasis patients with PTPH are older with additional symptomatology. Serum calcium, alkaline phosphate, parathyroid hormone levels were predictors of PHPT. Nephrocalcinosis, concomitant ureteric and renal stones and calcific pancreatitis were predictors of PHPT in urolithiasis patients.
Conclusion
PHPT should be considered as an etiologic factor in urolithiasis.

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P16

Impact of hyponatraemia in patients with fracture neck of femur

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Introduction
Hyponatraemia, defined as serum sodium <135 mmol/l is commonest electrolyte abnormality and is frequently encountered in elderly population. It is associated with osteoporosis and falls and an independent risk factor for fractures. The reported 1-year mortality for fracture neck of femur is between 20 and 35%.
Aim
To evaluate the impact of hyponatraemia on patients with fracture neck of femur compared to normonatraemia admitted to the hospital.
Methods
This is an observational retrospective audit to look at the incidence of hyponatraemia and outcomes such as length of stay, time to operation and mortality in patients admitted to acute hospital between October 2009 and March 2011. The admission sodium was used for statistical comparison.
Results
1050 patients were admitted with fracture neck of femur during this period. There were 23.1% of subjects with Na <135 mmol/l and 73.14% with normal sodium, defined as 135 to 144 mmol/l. Among the hyponatraemic group, 16.48% had Na 130–134 (mild), 4.95% Na 125–129 (moderate) and 1.72% Na <120 (severe). Patients with hyponatraemia Na <135 mmol/l on admission to hospital had significantly increased length of stay and delayed time to operation compared with normonatraemic group. They were also older than the patients with normal sodium. There was no difference with regards to within 30 days and over 30 days mortality in both groups. This may be due to the higher mortality associated with fracture neck of femur and in both groups the mortality was around 25% in keeping with other studies.

Table 1 Hyponatremia vs normonatremia (<135 vs 135–144)

Variable	Hyponatremia (*n*=243)	Normonatremia (*n*=768)	*P*
Age – mean (S.D.)	81.53 (10.85)	79.43 (15.19)	0.046
Gender – men (*n* %)	63 (25.93)	229 (29.82)	0.24
Length of stay – median (IQR)	25 (11, 49)	21 (11, 40)	0.046
Time to operation – median (IQR)	2 (1, 5)	1 (1, 3)	0.048
Overall mortality	62 (25.51)	203 (26.43)	0.78
Mortality with 30 days	29 (11.93)	99 (12.89)	0.70
Mortality over 30 days	33 (13.58)	104 (13.54)	0.99

Conclusion
There was high prevalence of hyponatraemia in patients with fracture neck of femur. The length of stay was significantly longer by 4 days and the time to surgery was delayed by 1 day in hyponatraemic subjects. Early identification and management of hyponatraemia and associated medical conditions may help to improve the clinical outcomes.
Declaration of interest
Invited speaker for Otsuka company.
Declaration of funding
Received educational grant from Otsuka company towards study of hyponatraemia in fracture neck of femur.

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P17

Risperidone associated changes in prolactin and bone mineral density: a study from South India

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Background
Risperidone is a widely used antipsychotic, known to cause secondary hyperprolactinaemia. Hyperprolactinaemia is associated with erectile dysfunction, amenorrhea and reduced bone mineral density (BMD). However, there is insufficient information about the extent, severity and association between these side effects, particularly among the south Indian population.
Aim and objectives
To estimate the prevalence of erectile dysfunction and amenorrhea, hyperprolactinaemia, subnormal BMD (osteopenia and osteoporosis), and vitamin D deficiency in patients taking Risperidone for more than 1 year. Also, to investigate whether erectile dysfunction (ED) or menstrual irregularity can be used as a proxy indicator of BMD loss in such patients, replacing dual energy X-ray absorptiometry (DXA) scan.
Method
Sixty-five patients (32 men and 33 women) (mean age (S.D.)=29.6 (6.5) years) receiving Risperidone as the only prolactin raising medication for minimum period of one year were studied. The history of erectile dysfunction and menstrual irregularities, BMD measurement of their lumbar spine and hip, serum prolactin and serum 25-hydroxy vitamin D levels were assessed.
Results
Erectile dysfunction was reported by 44% men ($n=14$) and amenorrhea by 24% women ($n=8$). The prevalence of Hyperprolactinaemia (>25 ng/ml) in women and men (>20 ng/ml) were 84.4 and 78.8% respectively. Subnormal BMD was found in 50% of the subjects. Furthermore, 30% subjects had vitamin D deficiency (<20 ng/ml) and 61% had vitamin D insufficiency (<30 ng/ml). A statistically significant association was observed between subjects having either ED or MD with subnormal BMD as compared to those not having them (OR 3.71; 95% CI: 1.23–11.24. $P=0.02$).
Conclusion
These results suggest that patients on long term Risperidone are at a greater risk of developing hyperprolactinaemia, reduced BMD and vitamin D deficiency although multiple contributory factors or mechanisms may be responsible. However, these findings have to be corroborated in a larger study population.

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P18

Predictors of low bone mineral density in an Irish cystic fibrosis (CF) cohort

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Increased life expectancy in patients with CF has brought about novel challenges in their care. Osteoporosis in CF is associated with significant morbidity and is an exclusion criterion for lung transplantation. Recent evidence suggests an association between dysglycaemia and low bone mineral density (BMD).
We aimed to determine predictors of bone loss in a cohort of CF patients attending a tertiary referral centre and, in particular, if dysglycaemia is linked with low BMD.
We performed a retrospective review of patients included in our hospital CF database. Data recorded included patient demographics and anthropometric characteristics, biochemistry, BMD as measured by dual-energy X-ray absorbimetry (DXA), lung function, medication prescribed and number of hospitalisations. The impact of patient characteristics on BMD was analysed by χ^2 test for discrete variables and Student's t-test for continuous variables. Spearman correlation between patient variables and Z scores was calculated.
Complete data was available for 92 patients. Median age was 25 ± 5 years and BMI 20.9 ± 4 kg/m^2. Sixty-three patients (68%) had a Z-score ≤ -1 of whom 17 had a Z-score ≤ -2.5. Fifty-five had normal glucose tolerance, 12 had impaired glucose tolerance and 29 CF-related diabetes with HbA1c of 5.4 ± 0.5, 5.9 ± 0.4 and 7.7 ± 1.8% and Z-scores of -1.4 ± 1.2, -1.4 ± 1.0 and -1.4 ± 1.5, respectively. Lower Z-scores were associated with poor lung function, low body weight and higher rates of hospitalisation and antibiotic use but not with dysglycaemia.
Despite recent evidence suggesting an association between dysglycaemia and bone loss in CF, we found no such association in our Irish cohort. Global disease severity and not dysglycaemia is the better predictor of bone loss in CF.

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P19

Comparison of different measures of urinary calcium excretion in primary hyperparathyroidism

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Patients with primary hyperparathyroidism (PHP) should have assessment of urinary calcium excretion as part of routine work up. This helps in the differential diagnosis of PHP in that urine calcium is low in familial benign hypocalciuric hypercalcaemia. Possible measures of calcium excretion include 24 h urine collection, spot sample for urine for urine calcium concentration and calcium/creatinine ratio (UCa/creat), and fractional excretion of calcium (FrExCa=urine calcium×serum creatinine/serum calcium×urine creatinine). 24 h urine collections, although perceived as gold-standard, are notoriously difficult to carry out reliably while FrExCa requires simultaneous blood and urine testing and a further calculation based these results. Spot urine for urine calcium concentration and UCa/creat on the other hand is easy to collect and requires no further calculation. It is not established whether any of the three measures of urinary calcium excretion (urine calcium concentration, UCa/creat and FrExCa) correlate with one another. Close correlation between any two would suggest that there was little point in measuring both.
We used a database of 51 confirmed cases of PHP to collect simultaneous urine and blood samples and measured urine calcium concentration, UCa/creat and FrExCa for the 51 datasets. Using Excel software we estimated correlation coefficients between the three.
The following correlation coefficients were obtained. Urine calcium concentration and UCa/creat $r=0.431$, Urine calcium concentration and FrExCa $r=0.385$, and UCa/creat and FrExCa $r=0.961$. UCa/creat and FrExCa were very closely correlated with each other, but both measures were weakly correlated with urine calcium concentration. Our long term objective will be to test whether any of these three measurements can be used to reliably differentiate between PHP patients and the normal population. With this in mind, since UCa/creat and FrExCa are so closely correlated there would seem little point in measuring both, and because FrExCa requires both a blood sample and a further calculation then UCa/creat would be the preferred option. We intend therefore to test whether urine calcium concentration and/or UCa/creat can be used to differentiate between PHP patients and the normal population.

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P20

Metabolic sydrome in women with osteoporosis on bisphosphonate therapy

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Metabolic syndrome (MetS) is known to be associated with low levels of vitamin D. The association of vitamin D in postmenopausal women with MetS and osteoporosis has not been investigated. The aim of our investigation was to assess presence of MetS in postmenopausal women treated for osteoporosis.
Metods
Fifty-nive women treated with weekly alendronate, vitamin D (Fosavance 5600, MSD) and calcium for 1 year were evaluated. The following parameters were assessed: DEXA at the level of lumbar spine and hip, parathyroid hormone levels (PTH), 25 hydroxycholecalciferol (25OHD3), lipids and glucose levels, blood pressure, waist circumfrence and BMI.
Results
Mean age of subjects was 65.6 ± 8.1 years, indicating late start of osteoporosis treatment. After 1 year of therapy, osteoporosis was still present in 37, osteopenia in 17, while five women attained normal bone mineral density. Metabolic syndrome was found in 38 women. The mean concentration of 25OHD3 in women with metabolic syndrome was 59.1 ± 23.3 nmol/l, while it was 114.25 ± 35.6 nmol/l ($P<0.05$) in those with no MetS. Levels of PTH did not differ between the groups. Metabolic syndrome was present in 45.2% of women with osteopenia, 54.8% of those with osteoporosis and in three women with normal

bone mineral density, after 1 year of treatment of osteoporosis. Metabolic syndrome was not present in 71.4% of women with osteoporosis, 28.6% of them with osteopenia and in two women with normal bone mineral density while of weekly alendronate therapy.

Conclusion

Low levels of vitamin D in postmenopausal women with MetS may compromise treatment of osteoporosis. Evaluation of vitamin D levels prior to treatment and it's adequate supplementation in women with MetS, may improve outcome of treatment with alendronate.

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P21

The utility of neck MRI in the localisation of parathyroid adenomas in primary hyperparathyroidism

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Neck ultrasound (US) can facilitate minimally invasive parathyroidectomy by providing fine anatomical detail of enlarged parathyroid adenomas in patients with primary hyperparathyroidism (PHPT). The role of neck MRI in US negative/equivocal cases remains unclear. We aimed to evaluate the performance of neck MRI in parathyroid tumour localisation in the setting of negative or equivocal neck US.

We performed a retrospective review of a consecutive series of 35 patients (29 women) with a biochemical diagnosis of PHPT who had neck MRI performed over the last 5 years. All patients had either negative neck US or discordant results between the US and the ^{99m}Tc-sestamibi scintigraphy. Data recorded included biochemical, radiological, surgical and histological variables.

The median serum calcium 2.86 mmol/l (range 2.66–3.55) and median PTH was 93 ng/ml (range 48–564). Seventeen patients underwent parathyroidectomy. Two patients did not achieve post-operative cure - one had confirmed multi-gland disease and the other had a failed neck exploration. Both patients had a negative MRI neck. The sensitivity of MRI was 47% in the whole group. However a positive MRI scan was 94% accurate in localising an enlarged parathyroid gland. Patient age, severity of hyperparathyroidism and adenoma weight were not predictive of a positive MRI scan.

In patients with primary hyperparathyroidism and non-diagnostic neck US or discordant imaging results, neck MRI can be useful with a high specificity although moderate sensitivity in adenoma localisation.

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P22

Renal replacement therapy to treat severe hypercalcaemic crisis: case series

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Background

The initial management of hypercalcaemia is well described: aggressive intravenous rehydration and subsequent intravenous bisphosphonates if required. Isolated case reports document the use of haemo/peritoneal dialysis and haemofiltration in the management of severe hypercalcaemia. We report three cases that required renal replacement therapy to treat severe hypercalcaemia.

Case series

A 21-year-old male presented with abdominal pain and vomiting. He was found to be hypercalcaemic (corrected calcium 5.00 mmol/l). A CT scan revealed pancreatitis. Parathyroid hormone (PTH) was 900 ng/l. He was promptly treated with intravenous fluids, pamidronate, calcitonin and haemofiltration to rapidly reduce his calcium level to excellent effect. He proceeded to urgent surgery; histology confirmed a 5×3 cm adenoma.

A 73-year-old female presented with a swollen leg secondary to deep vein thrombosis. Routine bloods revealed corrected calcium of 4.76 mmol/l (previously normal), and acute kidney injury (creatinine 478 μmol/l). Aggressive rehydration was delayed and after 24 h she required urgent haemodialysis to reduce her calcium level and control her fluid balance. PTH was 1644 ng/l. Urgent parathyroidectomy was performed; histology confirmed a dominant nodule on the background of hyperplasia. Renal function recovered after 16 days.

A 33-year-old male with known X-linked hypophosphataemia on long-term calcitriol and oral phosphate presented with a short history of nausea, vomiting and constipation. Corrected calcium was 4.61 mmol/l. He was treated with intravenous fluids, pamidronate, and the calcitriol was stopped. He represented 3 weeks later with similar symptoms and hypercalcaemia (3.8 mmol/l). PTH was 676 ng/l. Cinacalcet was started but seven days later he required haemodialysis for recurrent hypercalcaemia. He underwent urgent parathyroid surgery; histology confirmed four gland hyperplasia.

Conclusions

This series highlights the important role of renal replacement therapy in the management of severe hypercalcaemia: in medical optimisation for urgent parathyroid surgery, in the presence of direct complications of the profound hypercalcaemia, or in treatment resistant cases.

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P23

Audit of cost-saving following introduction of investigation protocol for primary hyperparathyroidism

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This group has previously audited the effect of a protocol for investigation of primary hyperparathyroidism (PHP); the protocol stating that only patients meeting criteria for parathyroidectomy should have parathyroid imaging carried out. This showed a reduction in the number of radiological investigations (USS, CT, MRI and sestamibi scans) done in patients with primary hyperparathyroidism following introduction of this protocol.

The aim of this audit was to compare the cost of investigating those prior to implementing the protocol, to the cost afterward.

47 cases of confirmed primary hyperparathyroidism were reviewed; 22 of those before the protocol introduction (December 2008), 25 after. We liaised with our local radiology department, and they were able to give us costings (including personnel, equipment use and ancillary costs i.e. contrast media) for each of the investigations utilised in the pre-operative assessment of these patients. An USS neck was found to cost £54.93, CT neck - £160, MR neck £368.73 and sestamibi scan £400.00. By calculating the number of investigations eliminated via the protocol, we used this and the costings to derive a figure for the savings.

Prior to the introduction of the protocol, five patients who were not considered for parathyroidectomy had imaging studies, comprising three ultrasound scans, four sestamibi scans, one CT and one MRI scan; a total cost of £2294.34. Since the protocol has been in use, two patients who have not been referred for surgery have had localisation scans – two ultrasounds and one sestamibi scan – total cost of £509.86.

Introduction of a protocol to rationalise radiological investigation of PHP resulted in a saving of £1784.48 by reducing the number of inappropriate investigations.

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P24

Effect of bone mineral density in patients with non-functioning pituitary adenoma

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We look into total 67 patients with non-functioning pituitary adenoma and review their bone mineral density in association with age and gender, effect of radiotherapy and hormonal replacement.
Age range
5.97% (4 patients) are between 18 and 40 years, 43.28% (29 patient) between 41 and 60 years and 50.74% (34 patients) are between 61 and 86 years.
Gender
61.19% (41 patients) are male and 38.8% (26 patients) are female.
Radiotherapy
59.7% (40 patients) received Radiotherapy. Among them 70% (28 patients) had normal BMD, 22.5% (9 patients) had osteopenia and 7.5% (3 patients) had osteoporosis.
16.41% (11 patients) developed osteopenia and 1.49% (1 patients) had osteoporosis even without radiotherapy.
Treatment
29.85% (20 patients) received growth hormones. Among them 19.4% (13 patients) had normal BMD, 8.95% (6 patients) had osteopenia and 1.49% (1 patients) had osteoporosis 50.74% (34 out of 67) received sex hormone replacement. Among them, 32.83% (22 patients) had normal BMD, 16.41% (11 patients) had osteopenia and 1.49% (1 patients) had osteoporosis.
8.95% (6 patients) needed vitamin D supplement. Among them 5.97% (4 patients) had normal BMD and only 2.98% (2 patients) had osteopenia.
61.19% (41 patients) were taking hydrocortisone. Among them, 29.85% (20 patients) had normal BMD, 23.88% (16 patients) developed osteopenia and 5.97% (4 patients) had osteoporosis.
Conclusion
Majority of the patients are in the age range of 61–86 years and most of them are male. Radiotherapy, hormonal deficiencies or hormonal replacement did not influence outcome of BMD.
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Conclusion
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P25

Proximal myopathy: a diagnostic dilemma

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Vitamin D deficiency with secondary hyperparathyroidism is common in South-east Asia. In contrast, primary hyperparathyroidism is relatively rare. We present a case of severe proximal myopathy with significant diagnostic delay.
A 23-year-old lady presented with a 2 years history of lower back pain, radiating to both groins and upper thighs associated with recurrent falls. Her pain and weakness progressed insidiously leading to difficulty standing or walking independently. She had been admitted under orthopedics and neurology at different hospitals where MRI spine/thigh and nerve conduction studies were normal, but serum calcium was low. She was diagnosed with 'lumbago' and sciatica. Management was four glasses of milk/day, ultrasonic massage and physiotherapy. Examination revealed proximal muscle weakness of the limbs, waddling gait, generalized bony tenderness, and bilateral genu valgus. Investigations showed 25OH-vitamin D <4 ng/ml, PTH 898 (15–65) pg/ml, phosphorus 1.5 (2.5–4.9) mg/dl, magnesium 1.9 (1.8–2.4) mg/dl, calcium 10.0 (8.5–10.1) mg/dl, CK 20 (21–15) IU/l, ALP 1959 (50–136) IU/l, 24 h urinary calcium 264 mg/dl, and 24 h urinary phosphorus 0.4 (0.4–1.3) g/24 h. She was commenced on vitamin D and phosphate. At follow-up, there was significant improvement in symptoms, particularly bony pain, ALP improved (1251 IU/l) but serum calcium and PTH increased to 10.9 mg/dl and 1161 pg/ml respectively. She was diagnosed with myopathy secondary to osteomalacia, primary hyperparathyroidism and hypophosphatemia. Neck USS and Sestamibi scan elucidated a $2.0\times1.3\times1.1$ c right inferior parathyroid adenoma. BMD revealed severe osteoporosis (Z-score L2–4: -4.7, femoral neck -3.9, forearm -5.7). She underwent Rt inferior parathyroidectomy. Recent biochemistry shows PTH 38.4 pg/ml and calcium 8.8 mg/dl on ergocalciferol 2000 IU/day and Calcium 1000 mg/day. Her symptoms subsided, except genu valgus, and she is independent and working.
Discussion
Myopathy has a wide spectrum of aetiological factors. Our patient had severe osteomalacia, hypophosphatemia and primary hyperparathyroidism. All of these conditions cause myopathy of variable severity. In our patient, it's difficult to determine the predominant aetiological factor. Myopathy due to metabolic causes is treatable and requires prompt diagnosis.

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P26

Audit of primary hyperparathyroidism management: do we adhere to the guidelines?

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Introduction
Primary hyperparathyroidism (PPTH) is a common referral to endocrine clinics with a clinical spectrum ranging from an asymptomatic state to a symptomatic disorder with or without end organ damage. We audited our management against the NIH guidelines which are endorsed by the Endocrine Society.
Methods
There is no outpatient coding system to correctly identify all patients with primary hyperparathyroidism. We included patient on radiology database who underwent imaging for the adenoma localization between April 2011 and March 2012.
Results
A total of 60 patients were identified, of which 68% were female. Mean age of the total cohort was 65 years. 12/60 (20%) were under 50 years of age and 48/60 (80%) were older than 50 years.
Only 23/60 (40%) had a 24 h urinary calcium measurement done and only 16/60 (27%) had a baseline DEXA assessment. Only 8/13 (62%) of those with a history of renal stones had a renal ultrasound (USG) with 8/45 (18%) with no history of renal stones had 'routine USG'. 52/60 (87%) of the patients underwent both parathyroid ultrasound and Sestamibi scan for adenoma localization.
9/12 (75%) of patients age 50 years or less had surgery. Of the remaining 3/12 (25%), one was waiting for surgery, one opted for conservative approach and one moved out of the country. In those >50 years, 37/48 (77%) met the criteria for surgery; 11/48 (23%) did not meet surgical criteria. In the former group, 22/48 (42%) had surgery, 12/48 (25%) were deemed surgical high risk or declined after tests, 3/48 were awaiting surgical review. All 31/31 (100%) patients undergoing surgery were cured.
Conclusions
Our audit highlights local variance in management and the need to adhere to the guidelines. We plan to discuss this in clinical governance meeting and formulate a performa for appropriate investigations for primary hyperparathyroidism.

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P27

The diagnosis of osteoporosis among subjects of Southern Indian origin above 50 years of age: the impact of the indian council of medical research vs caucasian bone mineral density reference standards

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Introduction
In the year 2010, the Indian Council of Medical Research (ICMR) has published a normative data for mone mineral density (BMD) measured by dual energy X-ray absorptiometry (DXA) scanning. However, its impact on the diagnosis of osteoporosis when compared to currently used Caucasian database has not been analysed.
Objectives
To study the effect of the newly generated ICMR database (ICMRD) on the diagnosis of osteoporosis compared with the Hologic DXA-4500 series database (HD) in subjects above the age of 50 in a tertiary care centre from South India.

Methods
A cross-sectional study of DXA scans performed between January 2009 and December 2011 was done. The reference standards of BMD obtained in the ICMR study for the hip and spine were used to recalculate the *T*-scores, and their agreement with HD in the diagnosis of osteoporosis was ascertained.
Results
A DXA scan of the lumbar spine in 4427 subjects (M:F = 544:3883) and hip in 3677 subjects (M:F = 467:3210) were analysed. The mean age (S.D.) of the subjects was 61.3 ± 8.4 and 59.7 ± 7.5 years in males and females respectively. Osteoporosis at the spine and hip were diagnosed in 1859 (42.7%) and 404 (11.4%) subjects by HD and in 1186 (27.7%) and 296 (8.3%) subjects by ICMRD respectively. A significant agreement existed between the two databases for the diagnosis of osteoporosis at the spine ($k = 0.657$; $P < 0.001$) and hip ($k = 0.808$; $P < 0.001$). A greater proportion was diagnosed as having osteoporosis with HD over ICMRD (at lumbar spine by 35.1% and hip by 27.1%).
Conclusion
Though a larger proportion of subjects were diagnosed with osteoporosis using HD over ICMRD at both sites, there was a significant agreement between the two methods for the diagnosis. However, further studies are required to denote as to whether a similar degree of agreement exists for the diagnosis of osteoporosis in those subjects with fractures.

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P28

Not the end of brown tumours: three cases within 12 months

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A 37-year-old woman presented with a short history of left arm pain. X-ray indicated a lytic lesion of the scapula. Calcium was elevated at 3.25 mmol/l (normal 2.2–2.6) with parathyroid hormone (PTH) 936 pg/ml (5–70). Upon questioning she reported nocturia, polydipsia and dyspepsia. Magnetic resonance imaging revealed brown tumours in the scapula, clavicle and hand. A superior parathyroid adenoma was excised with normalisation of calcium. Bone biopsy of the scapular lesion revealed giant cells in keeping with a brown tumour.
A 52-year-old man presented with left hip pain, and mild nocturia and polydipsia. Radiographs demonstrated a pathological fracture of the femoral neck due to a lytic lesion, which upon biopsy revealed a brown tumour. He had primary hyperparathyroidism (calcium 3.44 mmol/l, PTH 835 pg/ml) due to a right inferior parathyroid adenoma.
A 66-year-old man presented with nausea, headache and a lump on his shin. He had primary hyperparathyroidism (calcium 3.9 mmol/l, PTH >1500 pg/ml). X-rays were in keeping with a brown tumour of the left tibia. A left inferior parathyroidectomy was performed and the tibial mass has decreased in size post operatively.
Brown tumours result from excessive osteoclastic resorption of trabecular bone followed by reparative bone deposition causing expansion beyond the usual shape of the bone. They are a feature of advanced hyperparathyroidism and are seldom seen today. Despite significant hypercalcaemia however, all of these three patients had minimal symptoms, thus perhaps permitting development of this unusual complication.

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P29

A case of severe immobilization hypercacemia in a young patient on neurorehabilitation unit

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Introduction
Immobilization hypercalcemia (IH) is more common in children and adolescent due to increased rate of bone turnover and it usually develop 4–6 weeks post trauma but it can begin as early as 2 weeks and as late as six months. We report a patient who developed severer hypercalcemia after sustaining multiple fractures and immobilization.
Case
A 24-year-old man was admitted for neurorehabilitation after road traffic accident, sustaining a subarachanoid haemorrhage and multiple fractures. Four months after admission, he developed severe hypercalcemia of (3.63 mmol/l) and PTH was suppressed at 0.9 pmol/l. He had mild renal impairment initially. Thyroid and adrenal function, serum ACE, vitamin D and PTHrp were normal. Serum protein electrophoresis and skeletal survey was normal which excluded multiple myeloma. CT Thorax, abdomen and pelvis and bone scan were unremarkable except hypertrophic calcification around the hips. Normocalcaemia was achieved following intravenous fluids and zoledronic acid. After extensive investigation a diagnosis of immobilization hypercalcemia (IH) was made. Serum calcium was normal when last checked nine weeks after bisphosphonate therapy.
Discussion
Immobilization hypercalcemia (IH) is an under-recognized cause of hypercalcaemia. Risk factors include more severe immobilisation, pre-existing renal disease, and childhood/adolescence. Although our patient presented later after his trauma than average, the diagnosis should be considered in appropriate clinical setting after excluding other common causes of hypercalcaemia. Clinical suspicion of IH may reduce the need for unnecessary invasive procedures.

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P30

Cinacalcet treatment for hypercalcaemia in primary hyperparathyroidism

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Introduction
Cinacalcet acts at the calcium-sensing receptors on parathyroid cells to increase the sensitivity to circulating calcium concentrations. Studies have shown that this treatment is an effective means of managing hypercalcaemia in primary hyperparathyroidism.
Aims
The aim of this study was to determine the outcomes of patients who had received cinacalcet for at least 3 months for primary hyperparathyroidism.
Methods
We identified patients who had a diagnosis of primary hyperparathyroidism and who had been treated with cinacalcet for at least 3 months. Patients with renal disease were excluded from the study.
Results
12 patients were identified with a mean age 75.8 years, 10 males, 2 females. Two patients were admitted because of their hypercalcaemia. Two patients underwent parathyroid surgery after commencing cinacalcet which was subsequently discontinued.
Calcium and PTH data
Pretreatment PTH range was 43–838 ng/l, median 150 ng/l. Pretreatment calcium range was 2.66–3.77 mmol/l, median 3.01 mmol/l. All 12 patients had an improvement in calcium levels with 6 patients achieving normocalcaemia. Three additional patients achieved calcium levels between 2.6 and 2.8 mmol/l. Median fall in calcium levels was 0.455 mmol/l (0.27–0.71 mmol/l). There was no correlation between initial PTH level and percentage fall in calcium levels. (correlation coefficient $r = 0.378$, $P = 0.225$) nor between initial calcium levels and percentage fall in calcium levels (correlation coefficient $r = 0.007$, $P = 0.9$).
Discussion
Our results are consistent with evidence in the literature that shows that cinacalcet results in biochemical improvement in calcium levels, with all subjects showing significant falls in calcium levels (between 9 and 20%) ($P = 0.002$). The absence of an association between initial PTH and calcium levels with the subsequent reduction in calcium levels suggests that improvements in calcium levels cannot be predicted based on these two measurements alone. We recommend this treatment as an alternative to surgery for primary hyperparathyroidism in patients with significant symptomatic hypercalcaemia unresponsive to conventional interventions.

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P31

AUDIT of patients referred for DXA scanning in a south Indian Tertiary care centre

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Introduction
Osteoporosis is a silent disease and fractures pose enormous medical and financial burden on ageing individuals. Also subjects of younger age are at risk for subnormal bone mineral density when they have systemic disorders like rheumatoid arthritis or on long-term medications like corticosteroids. Preventive measures can be initiated

if osteoporosis is diagnosed and treated before any fracture occurs. Even after the first fracture has occurred, there are effective treatments to decrease the risk of further fractures and associated morbidity and mortality.
Objective
To evaluate the current use of bone densitometry, referral pattern, and prevalence of osteoporosis in patients referred for DXA in a tertiary care centre.
Methods
Bone densitometry data of all the patients ($n=1480$) referred for DXA from 1 Aug to 31 Oct 2012 was collected. Their demography and biochemical data were recorded from Computerized Hospital Information Processing System network.
Results
The mean age ($\pm$s.d.) was 49.9 ($\pm$13.1) and 47% of subjects ($n=695$) was below 50 years. Steroid use was documented in 25% ($n=370$) of the patients, and among them, 12.5% had osteoporosis and 60% had osteopenia. In those above 50 years of age ($n=785$), 42% had osteoporosis and 44% had osteopenia. Calcium profile was evaluated in only 65.6% and vitamin D in 75.6% of the patients. Vitamin D deficiency (<20 ng/ml) was present in 61%. The most common referrals were from Rheumatology (28%), followed by Internal Medicine (25%), Endocrinology (21%) and Orthopedics (16%). The most common reasons for referral were for postmenopausal state and inflammatory arthritis.
Conclusion
Utilization of DXA scanning as a tool for diagnosis of osteoporosis is restricted to only few specialties. A large section of women and men with osteoporosis who need preventive measures, vitamin D and calcium supplements still remain undiagnosed.

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P32

Audit of management of patients with primary hyperparathyroidism in district general hospital

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Aim
The aim of this audit was to evaluate our adherence to current recommendations by Third International Workshop on the Management of Asymptomatic Primary Hyperthyroidism.
Method
We audited 50 cases from our database of patients with PHPT diagnosed between 2007 and 2012.
Results
21/50 (42%) were referred for surgery with 19/21 operated. All referred patients had SESTA MIBI preoperative localisation scan. 19/21 had USS in addition. 28/50 (56%) patients were managed medically. 3 patients, who were not considered for surgery had localisation imaging done. 10/50 (20%) had calcium level $>$ 2.85 mmol/l. 8/10 were referred for surgery, 1/10 was not a surgical candidate and in 1/10 'watch and wait' strategy was adopted. 6/50 (12%) patients were <50 years, 4/6 were referred for surgery, 1/6 was thought to have no indications and 1/6 had severe mental disability.
Renal imaging was not routinely done with 58% patients having renal US done as part of work up. 8/29 (28%) patients had stones/nephrocalcinosis. 5/8 were referred for surgery. DEXA scan was requested in 35/50 (70%) patients. 5/35 (14%) patients had osteoporosis, 4/5 had parathyroid surgery. The remaining patient not referred for surgery was thought to have no surgical indication. 15/50 (30%) patients had no 24 h urinary calcium levels done as part of initial work up.
Conclusion
The majority of patients sampled were managed medically however a small number of patients who were not considered suitable for surgery had unnecessary localisation imaging. Imaging for complications of osteoporosis and renal stones was sub-optimal, occurring in 70 and 58% of patients respectively, suggesting that patients eligible for surgery may have been missed. In addition a small number of patients fulfilling criteria for surgical treatment were managed medically.

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P33

Osteogenic malignancy and severe vitamin D deficiency (osteogenic osteomalacia)

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Introduction
Vitamin D deficiency is increasingly being recognized as a metabolic disorder in temperate climates with various bone, cardiovascular and systemic manifestations. However topical, it is important to exclude tumour induced osteogenic osteomalacia as a possible aetiology in severe vitamin D deficiency when other risk factors (vegetarian diet, ethnicity, etc.) are absent. Early diagnosis of malignant tumors could be life saving and their resection may make this vitamin D resistant syndrome respond to treatment. We would like to introduce and discuss the importance of fibroblast growth factor-23 (FGF-23) as a tumor marker and a possible paraneoplastic substance.
Case report
A 58-year-old Caucasian man presented to his general practitioner with severe back pain and very low vitamin D3 levels (<10 nmol/l). Alkaline phosphatase was elevated at 160 U/l but calcium, phosphate and parathormone levels were normal. Over the next few months his calcium levels decreased further to a low of 2.06 mmol/l and phosphate to 0.76 mmol/l. He was prescribed vitamin D supplements and subsequently a Tc-99m bone scan revealed marked increased activity in the right proximal femur indicating an aggressive osteoblastic process, in-keeping with osteosarcoma. The femur was resected and a titanium implant inserted.
Discussion
Osteogenic osteomalacia is a very rare diagnosis with subtle manifestations which are considered a consequence of an underlying neoplasm, which can be very indolent. Undetected, the associated malignant tumors could metastasize. A high degree of clinical awareness and suspicion is required, particularly if the tumor is small along with the use of radiological investigations such as PET and Octreotide scans to detect occult tumours. FGF-23 is a member of the FGF family which is involved in phosphate homeostasis and skeletogenesis and may prove useful as a tumour marker to help exclude a malignant tumour in severe vitamin D deficiency.

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P34

Osteogenic malignancy and severe vitamin D deficiency (Osteogenic osteomalacia)

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Introduction
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Case report
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Discussion
Osteogenic osteomalacia is a very rare diagnosis with subtle manifestations, which can be a consequence of an underlying indolent neoplasm. Undetected, the associated malignant tumours could metastasize. A high degree of clinical awareness and suspicion is required, particularly if the tumour is small along with the use of radiological investigations such as PET and Octreotide scans to detect occult tumours. FGF-23 is a member of the FGF family, which is involved in phosphate homeostasis and skeletogenesis and may prove useful as a tumour marker to help exclude a malignant tumour in severe vitamin D deficiency.

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Clinical biochemistry

P35

A rapid and sensitive LC–MS/MS assay for the routine analysis of estradiol and estrone

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Introduction
Most clinical laboratories use immunoassays to measure estradiol despite limitations such as poor specificity, poor sensitivity and wide variability between different manufacturers' assays. LC–MS/MS assays overcome the issues of sensitivity and specificity however the methods reported in the literature often involve complex sample preparation and lengthy run times. We describe a simple, rapid assay for the simultaneous measurement of serum estradiol and estrone.
Methods
Sample (250 µl) was diluted with water after the addition of internal standards. After mixing, the diluted samples are transferred to the wells of a Biotage SLE+ plate. After extraction with MTBE, the ether is dried then extract is reconstituted with 100 µl of 40% methanol. Extract was extracted further using on-line solid phase extraction on a C18 cartridge by a Waters Acquity/OSM followed by a Waters TQS tandem mass spectrometer.
Results
The lower limits of quantitation for estradiol and estrone were 10 and 6 pmol/l respectively. The CV of the assay for estradiol and estrone concentrations of 125 pmol/l was $< 7\%$. Further the estradiol assay demonstrated a CV of 10% at 22 pmol/l and the estrone assay had a CV of 5% at 16 pmol/l. The average recovery for estradiol was 102% and estrone was 106%. The comparison with a commercial immunoassay gave the following equation: Immunoassay $= 0.94\times$ LC–MS/MS $+21$ pmol/l. The run time was 4.5 min per sample.
Discussion
We have developed a rapid assay for the LC-MS/MS measurement of estradiol and estrone which does not require derivatisation in the sample preparation. The assay is suitable for routine clinical use or for clinical trials. The assay demonstrated superior performance compared to immunoassays at lower concentrations making it more suitable for use in males and patients on aromatase inhibitors.

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P36

The search for 3-Epi-25-hydroxy vitamin D

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Background
The C-3 epimer of 25 Hydroxyvitamin D_3 (3-Epi-25OHD_3) is produced in the liver by the epimerisation pathway of 25-hydroxy vitamin D_3. It differs from 25OHD_3 in configuration of the hydroxyl group at the third carbon (C-3) position. Despite the fact that little is known regarding its clinical significance, concerns have been raised that isobaric interference may result in over-estimation of total 25OHD when measured by liquid chromatography tandem mass spectrometry (LC–MS/MS).
Objective
The aim of the study was to develop a chromatographic technique to resolve 3-Epi-25OHD_3 from 25OHD_3 and quantify by LC–MS/MS. The method was used to identify the presence of 3-Epi-25OHD_3 in samples received for 25OHD measurement at the Norfolk and Norwich University Hospital.
Method
Serum was precipitated with zinc sulphate and acetonitrile containing hexa-deuterated 25OHD_3. The extract was chromatographed using a 2.6 µm $100\times$ 2.1 mm I.D. pentofluorophenyl solid core particle column. Mass detection and quantification were performed by positive electrospray ionization with MS/MS in multiple reaction monitoring mode.
Results
The method was able to fully resolved 3-Epi-25OHD_3 from 25OHD_3. The intraassay CV for the epimer were 6.3 and 4.1% at 25.4 and 62.1 nmol/l respectively; and interassay CV were 8.3 and 6.5% at 27.6 and 63.2 nmol/l, respectively. In the patient samples tested, we found an average of 4.2 nmol/l of 3-Epi-25OHD_3 present. One patient sample had a 25OHD_3 of 187 nmol/l that was shown to contain 141 nmol/l of 25OHD_3 and 44 nmol/l of 3-Epi-25OHD_3.This patient was receiving a high dose of vitamin D supplementation.
Conclusion
We have developed a method for 3-Epi-25OHD_3 that is quick and robust for routine use. The method can be easily adapted into any existing LC–MS/MS system set up for 25OHD measurement. Although the prevalence of 3-Epi-25OHD_3 was found to be low, 3-Epi-25OHD_3 can readily be separated to ensure accurate measurement of total 25OHD_3. This will facilitate further research into the clinical importance of the epimer.

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P37

Plasma metanephrine analysis by online solid phase extraction LC–MS/MS

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Background
Plasma metanephrine analysis is widely accepted as the test of choice for phaeochromocytomas and sympathetic extra-adrenal paragangliomas. It is important to analyse 3-methoxytyramine along with metanephrine and normetanephrine as 3-MT measurement has been shown to be useful in tumour localisation and also in determining whether metastasis has taken place. 3-MT analysis is challenging as the concentrations of 3MT of interest are very low, and the sensitivity of many assays is not sufficient to distinguish between normal and raised concentrations. We have developed a sensitive method for measuring metanephrine, normetanephrine and 3-methoxytyramine using an online solid phase extraction system coupled to a Waters Xevo TQS mass spectrometer.
After sample dilution with internal standard, deproteinisation is carried out using 10 K centrifugal filters. 75 µl of deproteinised sample is loaded onto a Waters Acquity OSM system, using weak cation exchange cartridges for further on-line sample clean-up. Chromatography is carried out on a Waters Hilic 3 µm $2.1\times$ 50 mm column, and mass spectrometry is performed on a Waters Xevo TQS mass spectrometer.
The recovery of samples from the centrifugal filters was $>95\%$ for all 3 analytes at a concentration of 3 nmol/l. The LLOQ was 0.0375 nmol/l for metanephrine, and 0.075 nmol/l for normetanephrine and 3-MT. The assay was linear up to 30 nmol/l for all analytes, and a good correlation was shown between this assay and the assay currently in use in our laboratory for metanephrine and normetanephrine, with an r^2 value >0.99.
We have developed a sensitive assay for plasma metanephrine analysis, which correlates well with our current assay for metanephrine and normetanephrine. The LLOQ of 3MT is 0.075 nmol/l, which will enable us to distinguish between normal and raised levels, something which has not been possible with previous assays.

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P38

Development of an inductively coupled plasma-mass spectrometry method for measurement of urine iodine and assessment of iodine status in subclinical hypothyroidism

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Iodine deficiency may lead to reduced thyroid hormone production and ultimately hypothyroidism. The UK has previously been considered to be iodine sufficient, however recent evidence suggests the UK may be iodine deficient. Iodine status can be assessed in several ways, including measurement of urinary iodine excretion, for which inductively coupled plasma-mass spectrometry (ICP-MS) is considered the gold standard method.
An ICP-MS method for determination of urine iodine was developed using an Agilent $7700\times$ instrument with auto-sampler and integrated sample introduction system. Published methods showed discrepancies in the choice of diluent therefore this was optimised. The final diluent was alkali based (tetramethylammonium hydroxide) including tellurium 125 as internal standard. Following method validation, iodine concentration was measured in spot urine samples from 203 individuals (18–70 years) enrolled in a local study recruiting patients with subclinical hypothyroidism (TSH >5 mU/l). These individuals had known thyroid function tests results at the time of urine collection.
Method validation studies demonstrated that the assay allowed accurate, precise, and sensitive quantification of iodine. Intra- and inter-assay coefficients of variation were 3.6 and 3.0% respectively. The limit of detection was 2 µg/l and the limit of quantitation was 1 µg/l. Minimal carryover was observed, and linearity of dilution was demonstrated. Measurement of urine iodine in 203 individuals revealed a median concentration 93 µg/l (range 8–3340 µg/l), consistent with mild iodine deficiency according to World Health Organisation classification. No correlation was found between urine iodine concentration and TSH or free T_4 in this cohort.
An ICP-MS assay for analysis of urine iodine has been successfully developed and validated. Use of this assay demonstrated the presence of mild iodine

deficiency in a local cohort, consistent with a previous report suggesting iodine deficiency in the UK. This assay may have utility for further investigating iodine deficiency in the local population.

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P39

Cross-reactivity of ten recombinant insulin preparations in the Abbott Architect Insulin immunoassay

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Preparations of recombinant insulin and insulin analogues are used in the treatment of insulin-dependent diabetes. Recombinant insulin that corresponds to the human sequence might be expected to cross-react extensively with insulin assays. Insulin analogues contain modifications that alter their action profiles and may therefore interact unpredictably with detection antibodies. In the differential diagnosis of hypoglycaemia it is important to be aware of the extent of the cross-reactivity of different insulin preparations with the insulin assay in use.

Ten commercial insulin preparations were investigated: Actrapid, NovoRapid, Levemir and Insulatard (Novo Nordisk); Humulin S, Humalog, Humulin M3 and Humulin I (Lilly); Apidra and Lantus (Sanofi-Aventis). A measure of cross-reactivity in the Architect Insulin immunoassay (Abbott) was obtained by means of an experiment designed to measure recovery in pooled human serum. The insulin preparations were sequentially diluted in insulin-depleted human serum to attain a stock material that was used to spike a pooled human serum (post-prandial) to final nominal concentrations of 20 and 100 mU/l. The Architect Insulin immunoassay is standardised against the WHO International Reference Preparation material 66/304.

All insulin preparations cross-reacted with the Architect insulin assay, but to varying degrees. The human sequence insulin preparations (Actrapid, Humulin S, Insulatard, Humulin I and Humulin M3) demonstrated similar levels of recovery (between 81 and 89%), indicating extensive cross-reactivity in the insulin assay. The insulin analogues (NovoRapid, Apidra, Humalog, Levemir and Lantus) demonstrated variable degrees of recovery. Apidra, a rapid-acting insulin, showed the lowest recovery at 12 and 14%, while Lantus, an ultra-long acting insulin, over-recovered at 127 and 140%, demonstrating significant cross-reactivity with the assay.

Commercially available insulin preparations cross-react with the Architect Insulin immunoassay. Interpretation of insulin levels in hypoglycaemia in the context of exogenous insulin administration requires knowledge of the cross-reactivity of such insulin preparations with the assay.

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P40

HPLC analysis on separation of BSA from dilute solution

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High performance liquid chromatography also called high pressure liquid chromatography (HPLC) is one of such sophisticated techniques by means of which we can do quantitative as well as qualitative measurement of different types of samples at a very low concentration even in the order of pictogram and nanogram level. The technology is an important one particularly in the field of Pharmaceutical Technology. Chromatography technique effects the separation of two or more component in a mixture. Our goal is to develop a unique method so that specific compound as mentioned can be identified in a single run from BSA protein qualitatively as well as quantitative from a BSA dilute solution. A Rheodyne model 7125 six port injection valve fitted with a 20 µl sample loop and a Novapak C18 column (150×39 mm, waters, USA) packed with 5 micrometer particles were used. The column was fitted with a Guard column (5 cm×4.6 mm) packed with the same packing material as in Novapak column. Single protein BSA can be easily be enriched or separated with a optimum chromatographic condition, at a flow rate of 0.8 ml/min, run time 8 min, injection volume 20 µl, mobile phase 0.9% NaCl +10 mM Tris buffer, pH 7.4. As a result BSA produced a peak at 1.406 min with the area 66 944 and the peak height was 6291. Comparing with the standard it can easily conclude that the peak was only for BSA sample.

Keywords: HPLC, BSA standard, BSA dilute solution.

Declaration of interest The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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P41

MSIA-SRM assay for parathyroid hormone and vitamin D binding protein: correlation with clinical immunoassay methods and application to clinical samples

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Parathyroid hormone is involved in calcium homeostasis through interactions with vitamin D. Because intact and truncated forms of parathyroid hormone (PTH) vary in their biological activity, assays that can accurately quantify the ratio of intact hormone to its fragments are of increasing significance in the diagnosis of endocrine, renal and bone diseases. Vitamin D and its metabolites circulate tightly bound to vitamin D-binding protein (DBP). Because DBP concentrations are altered in pregnancy, liver and renal diseases and also show genetic variations in different ethnic groups, total vitamin D in serum can be misleading. In addition, both calcium and vitamin D metabolites can decrease the secretion of PTH.

Previously, we developed multiplexed mass spectrometric immunoassay (MSIA)–SRM assays for PTH that allow quantification of four fully-tryptic monitoring peptides (that span the entire PTH sequence) and two semi-tryptic variant specific peptides[1]. Using this approach, it is possible to monitor intact PTH and also the degree of N-terminal fragmentation.

In this study, the objective was to develop a multiplexed, MSIA-SRM-based targeted assay for PTH and DBP. We applied this MSIA-SRM assay and a commercially available immunoassay to a cohort of 500 clinical samples from a variety of different patient groups including renal disease, cancer, vitamin D deficiency and other conditions that can affect calcium homeostasis. The results demonstrated excellent assay linearity ($R^2 = 0.90$–0.99) with sensitivity for analytes in the published clinical ranges and limits of detection in the pg/ml range. Comparison of the PTH MSIA – SRM assay with the commercial ELSA assay demonstrated good correlation in normal subjects but important differences in renal failure. There were also some unusual fragments seen in clinical samples, not previously reported in the literature.

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P42

Glycosylated linkers to generate long-acting GH tandems

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Background
The development of recombinant biologics has had a major impact on many diseases. However, most biologics are rapidly cleared from the body and therefore require frequent injection regimens. There is therefore a need for technologies that allow the half-lives of these molecules to be extended in a predictable manner.

Hypothesis
Increasing numbers of N-linked glycosylation motifs between two GH molecules leads to gradually increased half-life whilst retaining biological activity.

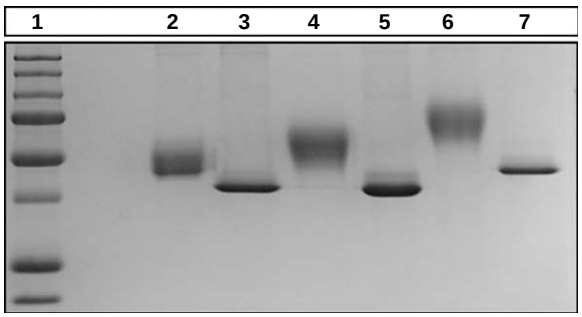

Figure 1 SDS–PAGE analysis of purified GH glycol-linker molecules containing increasing numbers of NAT (glycosylation motif) of QAT (control with no glycosylation). Shows increased MW with increased glycosylation.

Methods
A number of GH tandem molecules with linkers containing 2–8 NAT glycosylation motifs and their respective controls (in which N is replaced by Q in the sequence motif NAT) were cloned, sequenced and expressed in a CHO cell line. SDS–PAGE was used to verify increases in molecular weight and an in house dual luciferase reporter assay used to test bioactivity. Protein was purified using immobilised metal affinity chromatography (IMAC). Pharmaco kinetics (PK) were assessed using a rat model system.
Results
On increasing numbers of glycosylation motifs a concomitant increase in molecular weight (MW) as observed by SDS–PAGE (see Fig. 1) from 42 to 75 kDa ($n=8$ glycosylations). All GH tandems were purified using IMAC from suspension-adapted serum free cultures to >95% purity. PK was tested in tandem GH molecules with and without two glycosylation sites. The tandem without glycosylation was cleared rapidly and with an identical profile to monomeric GH whilst the tandem with glycosylation showed a fourfold slower clearance.
Conclusion
It is possible to increase the apparent MW of hormone tandems using glycosylated-linkers whilst maintaining bioactivity and that this also delays clearance of the molecule in rat model system providing a potential technology for generating long acting biologicals.

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P43

High pulmonary artery pressure is associated with BNP and NT-proBNP in lowlanders acclimatising to high altitude

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Background
We have previously demonstrated that the natriuretic peptides BNP and NT-proBNP rise with ascent to high altitude (HA). Both peptides are classically markers of congestive cardiac failure but have also recently been found to be associated with pulmonary hypertension at sea-level (SL). As pulmonary hypertension is central to the risk of high altitude pulmonary oedema we aimed to establish if there was any association between high pulmonary artery systolic pressure (PASP) and BNP/NT-proBNP at HA.
Methods
20 subjects from an expedition to Nepal (study 1) and 48 subjects from an expedition to Bolivia (study 2) were recruited and had BNP and NT-proBNP assayed and non-invasive assessment of PASP performed with ascent to 5150 m.
Results
BNP and NT-proBNP generally increased at altitude compared to baseline. PASP increased progressively with ascent. Generally, a PASP $\geq$40 mmHg (vs <40 mmHg) at various altitudes was associated with a higher BNP and NT-proBNP. For example, in study one at 5150 m those with a PASP $\geq$40 mmHg ($n=8$) vs those <40 mmHg had BNP of 54.5$\pm$36 vs 13.4$\pm$17 pg/ml ($P=0.012$). In study two those with a high PASP at 3600 m at rest ($n=6$) had higher BNP (37.5$\pm$38 vs 10$\pm$6.7, $P=0.003$) and NT-proBNP (370$\pm$413 vs 101$\pm$72, $P=0.003$).
Conclusion
BNP and NT-proBNP may serve as markers for elevated PASP, a central feature of high altitude pulmonary oedema, at HA. In this respect they offer the potential to facilitate early diagnosis and management, particularly with the availability of point-of-care testing.

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P44

Danazol cross-reacts in the Roche E170 testosterone assay

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The duty biochemist at Barts Health noted a testosterone result of 17 nmol/l on a 20-year-old female, measured by Roche E170 electrochemiluminescence immunoassay. Laboratory policy is that all female testosterone results >2.5 nmol/l by immunoassay (upper reference limit 1.92 nmol/l) are checked by LCMS to exclude analytical interferences. The LCMS result on this sample was below the limit of quantification (<0.5 nmol/l).
Clinical details were 'aplastic anaemia'. There was no previous testosterone result. LH and FSH were 8.6 and 6.3 IU/l respectively. Patient's notes revealed that she was on danazol to treat anaemia secondary to Dyskeratosis Congenita, at a dose of 200 mg alternating daily with 100 mg.
The sample was diluted in steroid-free serum, and measured neat (confirming previous result) and at three dilutions. The dilution studies gave a linear regression of $y=1.01x-0.25$, with a correlation coefficient of $r^2=0.999$.
The sample was checked on an alternative platform; testosterone was 2.3 nmol/l (<4.5) on the Abbott Architect (one-step chemiluminescent microparticle immunoassay).
Danazol (200 mg capsule) obtained from Pharmacy was dissolved in methanol (10 ml), spiked into steroid-free serum to a final concentration of 988 nmol/l, and assayed for testosterone. On the Roche platform the result was 10.2 nmol/l (recovery of 1.04%). By LCMS, testosterone was reported as undetectable.
These data confirm that danazol cross-reacts with the reagent antibodies in the Roche assay. The manufacturer states that cross-reactivity of danazol is $\leq$0.5% at a concentration of 1000 ng/ml. Abbott state that danazol does not cross-react in their assay. Danazol (17α-ethinyl testosterone) is a derivative of ethisterone, and has significant structural homology with ethisterone and norethisterone. UKNEQAS have demonstrated that norethisterone (at pharmacological concentrations) interferes in the Roche testosterone assay. Users of immunoassay should be aware of interferences due to steroid drugs.

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P45

Persistent hyperparathyroidism following parathyroidectomy: can routine vitamin D replacement prior to surgery alter post-operative secondary hyperparathyroidism?

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Introduction
A persistently elevated parathyroid hormone (PTH) following parathyroidectomy is usually associated with vitamin D deficiency. We have previously demonstrated this to occur in ~60% of post-operative patients. We have examined the effect of routine vitamin D replacement pre-operatively on the proportion of patients with persistently elevated PTH post-operatively.
Methods
Data was collated retrospectively from our parathyroid database, including: patient demographics; pre- and post-operative biochemical results; operative data; and vitamin D treatment.
Results
126 consecutive patients undergoing parathyroidectomy for sporadic primary hyperparathyroidism were examined retrospectively (88 (70%) female). Pre-operative ultrasound resulted accurately localised 72% patients and nuclear medicine localised 64% allowing 82 patients (65%) to undergo targeted (minimally invasive) parathyroidectomy.
Median pre-operative serum calcium was 2.9 mmol/l (range 2.6–4.0), and median pre-operative PTH was 13.1 (range 4.5–849 pmol/l). 116 patients (92%) had a single gland excised. 70 of 88 (79.5%) patients who had vitamin D levels measured were found to be vitamin D deficient pre-operatively (<50 nmol/l) – median pre-operative serum vitamin D 35.1 nmol/l (range 7–127).
All patients received vitamin D (cholecalciferol 1000–2000 IU daily) at least 3 months prior to parathyroidectomy. There were no episodes of severe hypercalcaemia requiring more urgent surgical intervention. 97.6% patients were rendered normocalcaemic following parathyroidectomy (failure rate 2.4%). Of these, 45 post-operative patients (36%) were found to be vitamin D deficient. There were no episodes of prolonged hypocalcaemia post-operatively (Hungry Bone Syndrome).
Conclusions
A series previously presented by this unit reported 57% patients with persistently elevated PTH following parathyroidectomy. We now routinely replace vitamin D three months prior to parathyroidectomy, resulting in a reduction in this proportion. There were no adverse consequences of vitamin D replacement and replacement was successful in reducing post-op vitamin D deficiency. This reduction in secondary hyperparathyroidism is likely to relate to better bone health, which we are exploring.

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P46

Service review and demand management following clinical audit of urine free cortisol requesting at Barts Health NHS Trust

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Urine free cortisol (UFC) is analysed in 24 h urine collections in suspected Cushing's syndrome, and provides an integrated measure of cortisol secretion over the whole day. The aim of this audit was to review how clinically useful UFC results were, and whether this analytical service was still justified. The current automated immunoassay with manual sample preparation was time-consuming, expensive (disproportionate quality control / external quality assessment (EQA) sample analysis, and unused reagent frequently discarded), and prone to cross-reactant interference.

Requests from April 2010 to March 2011 were examined. Of 202 samples, 102 were requests from within the Trust, mainly from Adult Endocrinology (40%), 22% were EQA samples, and 28% were GP or referred samples.

Of internal requests, 34% were clinically appropriate (e.g. 'Cushing's syndrome', 'hypertension', 'relapse, known Cushing's'), and 17% were inappropriate (e.g. 'adrenal insufficiency', 'tall stature' (paediatric), 'renal stones'). Analysis was hampered, as 48% of requests had no clinical details. For 20% of requests a random urine sample was sent, the results of which are not clinically interpretable.

Clinical details, sample type, and whether the UFC result was raised or normal ($<$340 nmol/24 h) were considered in conjunction with the results of other biochemical and dynamic function tests to judge whether the test had 'added value' in the patient pathway. In only 10% of requests was the UFC test considered to add value. In a further 12% of samples UFC may have added value, although dynamic function testing was preferable (e.g. 48 h low dose dexamethasone suppression test).

Discussion with the clinical colleagues informed a consensus regarding when UFC is clinically required, and it was agreed to switch the assay to an LCMS method, which is more specific. Measures introduced following the audit have reduced requests by 30%, and prevented inappropriate samples and requests being assayed, reducing analyses by 80%.

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P47

Hyponatremia in patients with fractured neck of femur: short and medium term outcomes

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Hyponatremia is an electrolye disorder which is relatively common in hospitalised patients. Usually it is asymptomatic and mild but in severe cases has been associated with confusion and increased risk of morbidity and death. The aim of this study was to compare outcomes following fractured neck of femur (FNOF) in patients with and without hyponatremia and was facilitated by the population health datasets in Tayside obtained from the Health Informatics Centre, University of Dundee. Length of stay, readmissions and deaths were the main variables of interest. Also examined were the time from admission to surgery, the length of hospitalisation for recuperation/convalescence and the proportion of patients for whom normal serum was attained at discharge.

Patients were included in this study if between 1/1/2000 and 28/6/2011, they were admitted to hospital in Tayside, Scotland with FNOF. Individuals were diagnosed as having hyponatremia if, at the time of admission, there was a specific diagnosis of the condition or the first serum sodium recorded following admission was less than 125 mEq/l. Electronic databases containing primary and secondary care information were linked using a unique identifier for each patient. χ^2 methods, independent *t*-tests and non-parametric methods were used to compare baseline characteristics. Cox regression was used to estimate the unadjusted and adjusted hazard ratio (HR) of readmission and death associated with hyponatremia. Binary logistic regression was used to determine the effect size (odds ratio) of hyponatremia and other independent variables on binary outcomes.

Hyponatremia was associated with longer hospitalisation at index admission (30 vs 17 days, $P=0.003$) and increased risk of readmission for any reason (adjusted HR 1.60 (1.22, 2.11), $P=0.001$). There was some evidence of an increased risk of readmission for FNOF (adjusted HR 1.68 (0.99, 2.83), $P=0.052$) but no increased risk of death (adjusted HR 0.95 (0.75, 1.21), $P=0.661$).

Hyponatremia in patients with FNOF is a marker of longer hospitalisation and increased risk of readmission.

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P48

A comparison of serum chromogranin A measurement with 24 h urine and serum 5-hydroxyindole acetic acid measurement in patients with NETs

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Introduction

Chromogranin-A (CgA) is a 49 kDa protein of the granin/secretogranin family originating from dense-core secretory granules within cells of the diffuse endocrine system. CgA is currently the best available diagnostic biomarker for neuroendocrine tumours (NETs) with recent clinical guidelines advocating the measurement of CgA as part of the baseline biochemical profile in patients presenting with symptoms suspicious of a gastroenteropancreatic NET.

5-Hydroxyindole acetic acid (5-HIAA) is also utilised as a marker for patients with serotonin-secreting NETs. Most laboratories currently measure 24 h 5-HIAA excretion in urine samples. However, urine collections are cumbersome and may often lead to inaccurate assessment of 24 h 5-HIAA excretion. More recently, LC–MS/MS measurement of 5-HIAA in plasma and serum matrices has become available.

Method

The aim of this study was to compare serum CgA measurement to both serum and 24 h urine 5-HIAA measurement. We measured serum CgA, 24 h urine 5-HIAA excretion and serum 5-HIAA in paired samples from 20 patients with known 5-HIAA secreting neuroendocrine tumours. Patients receiving PPI therapy were excluded from the study.

Results

All results were expressed as a percentage of the reference range. Linear regression analysis of CgA and 24 h urine 5-HIAA gave a correlation coefficient of 0.92 with a corresponding Passing-Bablok regression equation of (urine 5HIAA)$=1.41\times$(CgA)-0.31. Linear regression analysis of CgA and serum 5-HIAA gave a correlation coefficient of 0.63 with a corresponding Passing-Bablok regression equation of (plasma 5HIAA)$=1.74\times$(CgA)-0.49.

Conclusion

We have demonstrated a strong correlation between 5-HIAA measurement in both 24 h urine samples and serum samples when compared to the current best available general NET marker CgA. Furthermore, in contrast to CgA measurement by immunoassay, 5HIAA measurement by LC–MS/MS is not susceptible to anti-reagent antibody interference, or the high dose hook effect and therefore offers greater analytical robustness.

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P49

An unusual case of chronic liquorice ingestion presenting as hypokalemic paralysis

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Long-term liquorice ingestion is a well-known cause of secondary hypertension and hypokalemia. However, its initial presentation with a very severe hypokalemia and rhabdomyolysis is exceedingly rare. We report a 46-year-old gentleman who presented with acute onset bilateral leg weakness. Medication included allopurinol, aminophylline, fluoxetine, gabapentin, omeprazole, and inhalers. He had a 40 pack year smoking history and drank alcohol heavily in the past.

On examination he had power of 2/5 in his legs and 5/5 in upper limbs. Severe hypokalemia of 2.6 mmol/l and raised creatinine kinase of 42 660 IU/l was noted. He had raised urinary potassium excretion at 104 mmol/24 h, suppressed aldosterone (61 pmol/l) and renin (0.3 pmol/ml per h)concentration and normal levels of cortisol and thyroid function.

Muscular weakness resolved with potassium replacement. Renal potassium excretion and hypokalemia normalised after several weeks. He represented with very similar symptoms and with potassium of 1.7 mmol/l. A detailed dietary history revealed that he consumed 1 bag (140 g) of liquorice daily for many years. He was diagnosed as suffering from Liquorice induced hypokalemia. 24 h urinary steroid chromatography profile was consistent with 11β-hydroxysteroid dehydrogenase two deficiency. He was discharged home with advice that he should stop consuming liquorice.

Liquorice induced hypokalemia and rhabdomyolysis is very rare. Liquorices' active ingredient, glycyrrhizic acid inhibits 11β-hydroxysteroid dehydrogenase, the renal enzyme which is responsible for conversion of cortisol to cortisone. As a result, renal mineralocorticoid receptors are activated by excessive cortisol,

which causes sodium and water retention and hypokalemia. In this patient a clear history of excessive liquorices ingestion, elevated renal potassium excretion, suppressed aldosterone and renin and biochemical confirmation of suppressed 11β-hydroxysteroid dehydrogenase deficiency confirmed the diagnosis. This emphasizes the importance of a detailed dietary history in unexplained hypokalaemia.

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P50

Role of urinary dopamine in investigation of phaeochromocytoma/ paraganglioma

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Introduction
There is controversy over which form of biochemical testing is best for screening for phaeochromocytoma/paraganglioma (PHAEO/PGL). Measurement of plasma metanephrines is the most sensitive test but is less specific and lacks widespread availability, and so many centres opt to measure 24-h urinary excretion of catecholamines and metanephrines. However, the standard 24-h urinary catecholamine profile also includes dopamine (DA), vanillylmandelic acid (VMA) and homovanillic acid (HVA).
The aim of the current study was to determine the additional value of measuring urinary DA in the diagnosis of PHAEO/PGL by reviewing patients who had elevated DA in isolation.
Methods
We reviewed 2161 (1118 cases) 24-h urinary catecholamine profiles analysed by HPLC and electrochemical detection at Crosshouse Hospital, Ayrshire between 2007 and 2010. We then reviewed the cases where an isolated DA elevation was seen.
Results
Ninety one samples (4.2%) demonstrated an elevation in 24-h urinary DA which corresponded to 51 cases (4.6%). 18 cases (1.6%) demonstrated elevated urinary DA in isolation. None of these had current evidence of PHAEO/PGL.
Conclusion
Elevated 24-h urinary DA excretion in isolation is unusual and of the cases we identified, there was no situation where this was of clinical relevance. These data would support the removal of urinary dopamine from the conventional urinary catecholamine profile (unless there is high suspicion of a DA secreting PGL) as in our series it had no impact on diagnostic yield and may lead to unnecessary investigations.

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P51

Liquorice induced hypertension and hypokalaemia

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Liquorice (scientific name *Glycyrrhiza glabra*) is historically used for gastrointestinal complaints. Now it is primarily used as a flavoring agent in the tobacco, confectionery and to some extent in the pharmaceutical and beverage industries. Excessive intake of liquorice may cause a primary hyperaldosteronism-like syndrome characterized by sodium and water retention, hypertension, hypokalaemia, metabolic alkalosis, low-renin activity, and hypoaldosteronism. We describe a 69 years old lady who presented through a General Practitioner with hypertension, dependent oedema and hypokalaemia. There was no history of diarrhoea or vomiting and diuretics or laxative use. She was an ex smoker and consumed a bottle of wine per week Her bloods showed severe hypokalaemia with potassium of 2.0 mmol/l. Her arterial pH was 7.50 with bicarbonate 42 mmol/l (metabolic alkalosis). Her serum renin and aldosterone were both low. Her Serum TSH, overnight dexamethasone suppression, ultrasound scan of the kidneys and 24 h urinary calcium were normal. Her history was reviewed once again and at that time it revealed that she had been taking liquorice in sweets for as long as she could remember. She stopped liquorice after that admission and was discharged home on oral potassium supplements Sando K, two tablets three times a day which she gradually reduced and was not on any potassium supplements for about two months when seen in out patients, She was normotesive, had no dependent oedema and her serum potassium was normal. The case emphasizes the importance of considering a detailed patient's history, which often may lead the treating physician to the correct clinical diagnosis.

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P52

Calcification of basal ganglia in chronic hypoparathyroidism

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Hypoparathyridsm and pseudohypoparathyroidsm are the common causes of pathological calcification in the brain though 0.3–1.5% cases are physiological. The clinical presentation of hypoparathyroidsm can vary with the calcium levels and chronicity of hypocalcaemia. We describe a 39-year-old female who had type one diabetes for the last 23 years. She was repeatedly hospitalised with collapse episodes thought to be hypoglycaemic though never proved. She also had primary hypoparathyroidism for the last 10 years and was on calcium and vitamin D supplements. Her adjusted calcium was 2.2–2.5 mmol/l. She had a cataract removed from the right eye and also had an early cataract in the left eye. Her fundal examination did not show any diabetic retinopathy. She also started to have tonic clonic seizures which initially were thought to be due to hypoglycaemic events but the capillary glucose was never documented in these episodes. She had slow speech, serpentine gaze and shuffling gait on examination. Her Chvostek's and Trousseau's signs were negative. She was sent for neuropsychological assessment to ascertain the cognitive impairment. There she had CT brain done which showed calcification in basal ganglia, centrum semi ovale bilaterally and also subcortical dense linear calcification bilaterally. She was seen by a neurologist and started on anticonvulsant treatment. Her seizures improved but no improvement in her cognition and gait abnormalities. This case emphasizes the importance of thinking about the whole spectrum of the disease even if the biochemical markers are stable on treatment.

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P53

A review of causes of hypomagnesaemia in hospital patients and its management

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An association between the use of proton pump inhibitors (PPIs) and profound hypomagnesaemia has been highlighted in a number of case reports. As the prevalence of this occurrence or cause remain unknown, we undertook a review of patients with significant hypomagnesaemia in our hospital with a particular focus on use of PPIs and management.
35 patients (21f; mean age (s.d.): 71.3 (14.6); median: 74 years) with significant hypomagnesaemia (defined as serum Mg <0.5 mmol/l, adjusted for albumin), for whom medical records were available, were identified from the laboratory computer. Information was extracted on serum Mg level, presentation, identified cause and treatment.
Serum Mg levels were 0.17–0.49 mmol/l (mean (s.d.): 0.30 (0.08); median 0.32); calcium levels were 1.35–2.61 mmol/l (mean (s.d.): 1.91 (0.33); median 1.93 mmol/l). 26/35 patients were prescribed PPI's – there was no difference between Mg or Ca levels for those on PPIs compared to the group as a whole.
Use of PPIs was deemed causative or contributory in 12 of 26, 7 of whom were switched to an alternative antacid, three were stopped and two continued. Other causes documented included GI loss in eight, chemotherapy in five, renal losses in three, alcohol abuse four, with no cause documented in 10.
There was lack of standardisation of treatment - for the group, 17, including nine in whom PPIs were considered causative, received IV replacement (duration: 30 min-4d; dose 20–96 mmol, mean (s.d.): 43.2 (27.5); median 40 mmol); two also received oral treatment (magnesium glycerophosphate 8 mmol tds). Of the remaining 19, four received oral replacement (three magnesium glycerophosphate 8 mmol tds, one unknown).
This short review shows PPI use to be common among patients with significant hypomagnesaemia, though it was considered as a contributory or causative factor in less than 50%. It also highlights a lack of standardisation in Magnesium replacement.

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P54

An audit on management of hyponatraemia in hospitalised patients

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Background
Amongst hospitalised patients, hyponatraemia is the commonest electrolyte abnormality with reported prevalence of about 25%. Its association with mortality, morbidity and increased length of stay is well recognised, including adverse fracture risk and falls with mild hyponatraemia in elderly. We audited the management of hyponatraemia in inpatients in a University hospital setting.
Methods
Over a 3-month period, amongst all in-patients, we identified those who had hyponatraemia (serum Na $\leq$ 130 mmol/l) from the in-house biochemistry database. Data on their demographics, management of hyponatraemia, length of stay and mortality was obtained and analysed.
Result
Of the 109 patients identified with hyponatraemia – their gender: 46% male; age: 75 $\pm$ 14 years (mean $\pm$ S.D.), with 60% being over 75 years age. The proportion who had serum Na $<$120, 121–125 and 126-130 mmol/l were 22, 24 and 54% respectively. Majority were managed in medical wards (91%) – acute medicine 19%, care of the elderly 27%, endocrine 19%, gastroenterology 10%, general medicine 8% and other medical specialities including respiratory medicine 8%. Only 21% had Endocrine specialist input. SIADH, fluid overload and drugs were attributed as cause for the hyponatraemia in 16%, 14% and 10% respectively and remaining 60% was due to other causes including vomiting and/or diarrhoea or where no clear cause was identified. The in-hospital mortality was 9.2% in the cohort, amongst whom 40% had serum Na $<$125 mmol/l. Of the 88.8% discharged, 36% and 35% had serum Na $\leq$ 130 mmol/l and $\leq$ 125 mmol/l at discharge. The average length of stay in those discharged was 12.4 days.
Conclusions
This audit demonstrates the potential deficiencies in the optimum management of hyponatraemia in inpatients setting. Large proportion had Na $<$130 mmol/l on discharge which is potentially associated with adverse outcomes especially in the high risk elderly population.

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P55

Impact of hyponatraemia in critically ill patients

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Introduction
Hyponatraemia, defined as serum sodium $<$135 mmol/l is one of the commonest electrolyte abnormalities seen in patients admitted to acute hospitals and is associated with increased morbidity and mortality. Impact of this condition is not adequately measured in critically ill patients admitted to intensive care unit. The aim of this observational study was to assess the incidence and outcomes of patients admitted to intensive care unit (ICU) in a UK based setting.
Methods
This was a retrospective observational study that looked into the incidence of hyponatraemia and outcomes such as mortality, length of stay, ventilator days, renal days in patients admitted to ICU between January 2011 and March 2012. Sodium levels were evaluated at four distinct time frames that included admission to hospital, admission to ICU, discharge from ICU and discharge from Hospital. Appropriate statistical tests were applied for comparisons with hospital and ICU admission Na.
Results
1289 patients were admitted during this time. Incidence of hyponatraemia at hospital admission as 27.5% and out of this 7.5% were moderately and severely hyponatraemic ($<$130 mmol/l). Incidence at ICU admission was 22.3% of which 3.3% were in moderate or severely hyponatraemic. Patients with hyponatraemia ($<$135) at presentation to hospital had increased APACHE II and ICNARC scores($P = <0.0001$). Patients with hyponatraemia at admission to ICU also had increased APACHE II and ICNARC physiological scores. Overall there was increased mortality (35.9 vs 21.2%, $P = <0.0001$) and ICU length of stay, and increased ventilator days.

Table 1 Hyponatraemia vs normonatraemia (Hospital admission sodium) ($<$135 vs 135-144)

	Hyponatraemia (n=353)	Normonatraemia (n=874)	P value
Age: mean (S.D.)	64.85 (16.35)	62.12 (17.65)	0.013
Gender: male (n %)	210 (59.49%)	520 (59.50%)	0.998
APACHE II score: mean (S.D.)	18.26 (6.75)	15.02 (6.42)	$<$0.0001
ICNARC model physiology score	18.24 (9.02)	14.73 (8.19)	$<$0.0001
ITU length of stay: median (IQR)	3 (2, 6)	3 (2, 5)	0.27
Status at discharge from ITU: alive	274 (77.6%)	756 (86.5%)	$<$0.0001
Hospital Length of Stay: median (IQR)	12 (6, 29)	11 (6, 23)	0.32
Status at discharge from Hospital: alive	231/273 (84.6%)	694/756 (91.8%)	0.001
Overall mortality %	122/340 (35.9%)	182/857 (21.2%)	$<$0.0001
Advanced respiratory days	0 (0, 2)	0 (0, 2)	
Advanced cardiovascular days	0 (0, 0)	0 (0,0)	
Renal days	0 (0, 0)	0 (0, 0)	
Advanced respiratory days $>$0	131 (37.11%)	302 (34.55%)	0.40
Advanced CV days $>$0	79 (22.38%)	156 (17.85)	0.068
Renal days $>$0	66 (18.70%)	79 (9.04)	$<$0.0001

Table 2 Hyponatraemia vs normonatraemia (ICU admission sodium) ($<$135 vs 135-144)

	Hyponatraemia (n=287)	Normonatraemia (n=894)	P value
Age: mean (S.D.)	65.98 (15.82)	61.76 (17.71)	0.0003
Gender: male (n %)	177 (61.67%)	525 (58.72)	0.38
APACHE II score: mean (S.D.)	17.40 (6.87)	15.32 (6.57)	$<$0.0001
ICNARC model physiology score	16.28 (8.98)	15.24 (8.44)	0.075
ITU length of stay: median (IQR)	3 (2, 4)	3 (2, 5)	0.032
Status at discharge from ITU: alive	236 (82.23%)	777 (86.91%)	0.048
Hospital Length of Stay: median (IQR)	13 (6, 28)	11 (6, 23)	0.46
Status at discharge from Hospital: alive	215/235 (91.49%)	703/777 (90.48%)	0.64
Overall mortality %	72/277 (25.99%)	193/873 (22.11%)	0.18
Advanced respiratory days	0 (0,1)	0 (0,2)	
Advanced cardiovascular days	0 (0, 0)	0 (0, 0)	
Renal days	0 (0, 0)	0 (0, 0)	
Advanced respiratory days $>$0	74 (25.78%)	323 (36.13%)	0.001
Advanced CV days $>$0	45 (15.68%)	160 (17.90%)	0.39
Renal days $>$0	33 (11.50%)	104 (11.63%)	0.95

Discussion
This study confirms findings available in literature on the increased morbidity and mortality in patients presenting with hyponatraemia to hospital and ICU. However, whether this caused excess mortality and morbidity in these patients is difficult to ascertain and prospective studies are required to evaluate the effect of correction of sodium levels on mortality and morbidity.
Declaration of interest
Invited speaker for Otsuka company.

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P56

Audit of inpatient management of hyponatraemia

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Introduction
Hyponatraemia is the commonest electrolyte disturbance occurring in 15–20% of inpatients (1), with significant clinical implications if mismanaged. We conducted a retrospective audit of our current management of hyponatraemia in our 1000 bedded trust.
Method
Data of patients with severe hyponatraemia (Na $<$125 mmol/l) admitted to hospital over 4 weeks (Aug 2011) was collected. Twenty randomly selected cases were reviewed focusing on initial assessment, management plan and associated morbidity.
Results
218 cases were identified during the study period. Among the 20 selected cases, the lowest admission sodium was 112 mmol/l, the mean age was 73 years (29–90) with 30% female, the cohort was also noted to have multiple co-morbidities (M= 4.56). Assessment of hyponatraemia was only mentioned in 10 (50%) cases on the consultant post take ward round. Fluid balance was recorded only in eight (44%) cases and no patient was assessed for postural hypotension. Aetiologies identified

were as follows; idiopathic (42%), drug induced (26%), SIADH (26%) and hypervolaemic causes (6%). Of the SIADH group the following investigations were performed; urinary sodium (60%), urine osmolality (60%) and serum osmolality (80%), thyroid function tests (20%) and 0900 h cortisol (14%). In relation to outcomes, fall in serum sodium was seen in 25%, with a rise evident in 65%, one case having >10 mmol rise in 24 h. The mean length of hospital stay (LOS) was 16 days (4–56 days) in comparison with a trust mean of 5 days, with 15% of prolonged LOS documented to be attributable to hyponatraemia. Mean sodium on discharge was 130 mmol/l (121–137 mmol/l), one death occurred post discharge (Na 122 mmol/l).

Conclusion

Hyponatraemia is commonly seen in patients with multiple co-morbidities. Management remains challenging and attributes to prolonged LOS. A clear local guideline is needed and is currently under development, to improve the standard of care.

Reference

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Clinical practice/governance and case reports

P57

Initiation and maintenance of mitotane as adjuvant therapy for adrenocortical cancer: a single centre experience

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Background

Mitotane is an adrenolytic chemotherapy, currently accepted as first line adjuvant therapy in adrenocortical carcinoma. Mitotane has a narrow therapeutic window. Serum levels of >14 mg/l are required to achieve a cytotoxic effect and levels of >20 mg/l are potentially toxic. There are two strategies for mitotane initiation: a low-dose regimen (3 g) and a high-dose regimen (increase to 6g/day over 4 days and reduce to 4.5 g/day after 10 days).

Methods

We conducted a retrospective review of consecutive mitotane use for adrenocortical carcinoma in a UK tertiary centre from 2006 to 2012. Mitotane initiation was completed using a nurse-led protocol.

Results

Initiation: Twenty patients were identified: 15 were prescribed the high-dose regimen and five the low-dose regimen. Eighty-five percent (17/20) achieved the therapeutic threshold (>14 mg/l). Mean time to reach therapeutic level was 2.2 months. Twenty percent (4/20) achieved therapeutic threshold in the first month. In total, seventy percent (14/20) achieved therapeutic levels within 3 months of initiation. Mitotane levels were performed 2-weekly for the first 3 months. The maximum dose used ranged from 3 to 8 g/day (mean 5.5 g/day).

Maintenance: Of the 20 patients initiated on mitotane, twenty-five percent (5/20) discontinued treatment because of side effects and/or intolerance (neurological and gastrointestinal symptoms). A further twenty-five percent (5/20) discontinued following disease progression with subsequent mortality. In total, seventy-five percent (15/20) of patients continued to maintenance phase (>3 months). Of these, seventy-seven percent of serum mitotane levels were above therapeutic threshold and fifty-three percent were within the range 14–20 mg/l. The mean maintenance dose of mitotane for this group was 4.8 g/day (range 2.5–8.0 g/day). Ten patients currently remain on mitotane.

Conclusion

Initiation of mitotane can be safely performed on an outpatient basis, using a nurse-led protocol. Seventy percent of patients in our series achieved mitotane levels above the therapeutic threshold within 3 months, comparing favourably to other published series.

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P58

De Novo HNF1b mutation as a cause for chronic treatment-resistant hypomagnesaemia

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A 29y female presented with an 8y history of hypomagnesaemia. It was noted incidentally when hospitalised with mumps-related pancreatitis. Subsequently symptomatic hypomagnesaemia, with headaches and lethargy, was treated with magnesium glycerol phosphate 4 mg TDS, but she remained symptomatic with occasional need of IV Mg^{2+}. It was thought that she was poorly compliant with her oral Mg^{2+} supplements. At presentation to our department for follow-up of her hypomagnesaemia, SeMg^{2+} was low (0.51 mmol/l) despite Mg^{2+} glycerol phosphate 4 mg TDS. Other biochemistry was normal including creatinine, vitamin D, total protein, PTH and cCa^{2+}. Urinary Na&K were normal and glucose negative. Urinary Ca^{2+} was low at 1 mmol/24 h (3–5 mmol/24 h). Urinary Mg^{2+} was (inappropriately) normal at 4 mmol/24 h (3–5 mmol/24 h). As the patient appeared to be losing Mg^{2+} from the renal tract, renal imaging with US and CT was performed, showing one large 2.8 cm and four 1.5 cm cysts in the left kidney, while the right was normal. The patient had a bicornate uterus. There was no significant family history. Referral to a geneticist lead to identification of a heterozygous whole gene deletion of HNF1-Beta (renal-cysts-and-diabetes syndrome: RCAD). Neither parent shared this mutation. HNF1b loss-of-function mutations are associated with MODY5, pancreatic insufficiency, renal cysts, hyperuricaemic nephropathy, single functioning kidney, gout, pancreatic atrophy and urogenital deformities (including bicornate uterus). In a paediatric population abnormal antenatal renal US is frequent with Mg^{2+}-wasting. HNF1B is important for the expression of FXYD2, which regulates ion transport in the distal convoluted tubule and inactivating mutations in FXYD2 also lead to hypocalciuria and hypomagnesaemia. The differential diagnosis of hypomagnesaemia includes diabetes mellitus, diuretics, platinum-containing chemotherapy, PPIs, EGF-antibodies, alcohol, hypercalcaemia and mutations in paracellin-1 (Mg^{2+}-wasting with hypercalciuria due to loss of tight junction, hinders Mg^{2+}-reabsorption in ascending loop of Henle), KCNA1 (K^{+}-channel which potentiates Mg^{2+}-reabsorption via transmembrane electrical potential) and EGF (stimulates Mg^{2+}-reabsorption of).

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P59

Outcomes of transsphenoidal surgery (TSS) for acromegaly

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Background

Pituitary surgery is the initial treatment for the majority of patients with acromegaly. The UK acromegaly register data (UK-AR-2) suggests that surgical remission rates vary widely, with a marked improvement since 2000. The aim of this study was to assess the outcomes of first TSS for acromegaly in our centre over the past 5 years.

Methods

We retrospectively analysed data for all acromegaly patients who underwent first TSS between 2007-2011. Biochemical remission was defined as normalisation of IGF1 and GH nadir <1 mcg/l post-GTT, or <2 mcg/l (random GH or series mean), at 3 months after surgery. Post-operative imaging was reviewed and data regarding pre and post-operative pituitary hormone deficiencies was collected.

Results

Two surgeons performed 22 first TSS operations for patients with acromegaly over the 5 year period (2.2 acromegaly operations /year per surgeon), including eight microadenomas, six intrasellar (IS) macroadenomas and eight extrasellar (ES) macroadenomas.

Post-operative remission rates for GH were achieved in 88% of microadenomas, 67% of IS macroadenomas and 25% of ES macroadenomas. The corresponding percentages for IGF1 were 75, 50 and 12.5%, and for both IGF1 and GH 62.5, 50 and 12.5%.

Post-operatively, six patients with macroadenomas developed new pituitary axis deficiencies, whilst five patients with pre-operative pituitary axis deficiencies recovered function post-operatively.

Comparative data

Mean biochemical remission rates following TSS for acromegaly in eight published series since 2000 are 78% for microadenomas, 59% for intrasellar macroadenomas and 25% for extrasellar macroadenomas. Most published series are from larger centres. Some studies only report outcomes for multi-modal therapy for acromegaly.

Conclusions

How many pituitary centres the UK should have for optimal outcomes, and whether centres should have one or two pituitary surgeons, remains an active

debate. Regular collection and reporting of surgical outcome data is essential to inform pituitary service provision.

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P60

How do medical students and doctors learn clinical endocrinology?

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Background
There has been little research into methods of learning clinical endocrinology, a speciality encompassing rare conditions and pattern recognition. Problems include limited patient exposure outside specialist centres and increasing pressure on doctors to manage their own educational needs. An understanding of learning methods in use across the spectrum of students and doctors will help to identify trusted resources and untapped techniques.
Aim
Pilot study to identify the usefulness of different learning resources employed by students and doctors currently engaged in endocrine practice.
Method
Participants completed a paper/online questionnaire indicating perceived usefulness of educational resources they had encountered. This was a prospective study using convenience sampling.
Results
77 responses were obtained: 57 third-year and 4 final-year students on endocrine placements, three foundation/CMT doctors, five registrars and 8 consultants. Lectures and textbooks were useful across all groups; but patient encounters were perceived as most helpful (100.0% of final year students and above). With increasing seniority, perceived usefulness of journals increased (25% of 3rd-year students, 50.0% of final-year students and 100% of registrars/consultants). Wikipedia was commonly-used by students (86.7% of 3rd-years found it helpful; 62.5% of consultants had never used it). Except for registrars (60% of whom found them useful), most participants had never used e-learning resources. 6.5% of participants had used podcasts and 26.0% had used apps for endocrine learning. Students are willing to use e-learning/podcasts if directed to these resources.
Discussion
Traditional resources and patient encounters remain important for learning. E-learning, apps and podcasts are relatively unexploited tools. Perceived usefulness is a helpful starting point for design and evaluation of grade-specific resources. Developing 'virtual patients' may benefit students and trainees, whilst senior physicians may value podcasts as a bite-sized method of delivering clinical developments. These will require evaluation for quality of learning as well as ease of use and popularity.

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P61

An unusual cause of Cushing's syndrome with secondary adrenal insufficiency

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A 20-year-old girl presented to the endocrine clinic with a history of three stone weight gain, and development of numerous purple striae over her lower abdomen, inner thighs and upper arms, gradually progressing over a 12-month period. The onset of symptoms had coincided with the initiation of contraceptive depot medroxyprogesterone acetate, which was discontinued two months prior to her presentation. Her 9 am cortisol was <50 nmol/l.
She had a background history of well controlled asthma, not requiring steroid inhalers for past two years. She was seeing the rheumatologists because of chronic thoracic pain and was on fentanyl patches. She had received two intramuscular steroid injections for this, last of which was two years previously. There was no other history of exogenous steroid use.
She had a short synacthen test that showed an undetectable baseline cortisol, rising to 263 nmol/l after stimulation. ACTH was 11 ng/l, with normal renin, aldosterone and baseline pituitary function. She was commenced on hydrocortisone 10 mg am and 5 mg pm and a glucagon stimulation test organised a month later showed a peak cortisol of 262 nmol/l. She was reviewed 4 months later, her Cushingoid features had significantly improved and a 0900 h cortisol was 265 nmol/l. Subsequently, a repeat glucagon stimulation test showed a peak cortisol of 604 nmol/l, consistent with a complete recovery of the HPA axis. She has been off hydrocortisone for last 2 months and remains asymptomatic.
Medroxyprogesterone acetate is a widely used potent progestational agent. It has low affinity for the glucocorticoid receptor resulting in low level glucocorticoid activity and may cause a combination of cushingoid appearance with secondary adrenal insufficiency when used in large doses, as in the treatment of cancer. To our knowledge this is the first case of such syndrome occurring with very small doses of medroxyprogesterone used for contraception purposes.

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P62

Management of intercurrent illness in adrenal insufficiency

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Several recent publications have advocated that patients with primary and secondary adrenal insufficiency (PAI and SAI) be given emergency injectable hydrocortisone to reduce the risk of adrenal crisis. There is a limited evidence-base for this recommendation and consequently the Edinburgh Centre for Endocrinology (ECE) does not routinely issue kits to its patients. This study assessed the frequency of hospital presentations in ECE's AI patients, and the severity of their condition on presentation. It also evaluated patients' knowledge of crisis-preventing behaviours, proportions with emergency hydrocortisone kits, and the influence of these factors on presentation rates.
Details on 81 PAI and 66 SAI patients and their presentations were obtained from patient records. Data such as blood pressure and biochemistry was collected, as well as reason for admission. 81 patients participated in a telephone questionnaire, part of which assessed their knowledge of 'sick day rules,' as defined by Pituitary Foundation literature. Patients were also questioned about emergency kits.
54.3% of PAI and 34.8% of SAI patients presented to A&E over a 6-year period. The trends suggested that PAI patients were more likely to be hypotensive, hyponatraemic, hyperkalaemic and have low bicarbonate, but these results were not statistically significant. PAI patients were, however, more likely to be hypoglycaemic (PAI 15.4%, SAI 0.0%, $P=0.048$). The commonest cause of presentation for PAI patients was gastrointestinal infection, and respiratory infection for SAI. Only 16% of patients knew to go straight to A&E when vomiting. 32% possessed emergency kits, but their frequency of presentation did not differ significantly from those without ($P=0.53$).
Improved education strategies may be required. Further studies should establish whether or not patients with emergency kits are less unwell on presentation than those without, and it may be useful to run a comparative study between ECE and another centre which does issue emergency kits.

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P63

A case of Hashimoto's thyroiditis induced coagulopathy

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A 26yr old man presented to haematology with a short history of easy bruising. There was no spontaneous bleeding. Past medical history was unremarkable. Family history of clotting disorders was negative. On examination there was significant lower limb bruising.
Initial investigations revealed platelets of 238 (150–450), prothrombin time (PT) of 12.0 (12.0–17.0 s) and elevated activated partial thromboplastin time (APTT) of 41.0 (24.0–38.0 s). The elevation in APTT alluded to an intrinsic pathway abnormality which was investigated through further clotting factor analysis. This revealed reduced levels of chromogenic factor VIII 0.46 (0.6–1.3 U/dl), factor VIII clotting assay 0.4 (0.6–1.3 IU/ml), von Willebrand Factor antigen 0.31 (0.7–2.0 IU/ml) and von Willebrand Factor activity 0.39 (0.7–2.0 IU/ml). Factor IX assay was normal at 0.63 (0.6–1.3 U/ml). This confirmed the diagnosis of von Willebrand disease.
On further questioning he complained of cold intolerance, dry skin and lethargy and there was a positive family history of hypothyroidism in an aunt. Clinical examination was unremarkable. Thyroid function was significantly deranged with unmeasurable free $T4$ <5.5 (9.0–19.0 pml/l) and grossly elevated TSH of 711.1 (0.4–4.5 mU/l). Anti-thyroid peroxidase antibodies were elevated at 3000 (0–135 U/ml) confirming the diagnosis of Hashimoto's thyroiditis. He was commenced on levothyroxine and the coagulopathy reversed (APTT 36.3 s, Factor VIII clotting assay 0.71 IU/ml, von Willebrand Factor Activity

0.78 IU/ml) as his thyroid function normalised.
This is a case of acquired von Willebrand disease due to Hashimoto's thyroiditis. It is the most common coagulopathy in hypothyroidism and is characterised clinically by easy bruising, epistaxis and mucosal bleeding and biochemically through elevated APTT, reduced Factor VIII and von Willebrand Factor. Normalisation of the thyroid axis usually reverses the coagulopathy negating the need for further treatment. Although a relatively uncommon complication of hypothyroidism it should be considered at diagnosis and coagulation status measured if clinically indicated.

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P64

Conn's syndrome with normal plasma renin aldosterone ratio

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Background
Conn's syndrome accounts for 35% of primary hyperaldosteronism. Elevated plasma aldosterone concentration to renin activity is widely used as a screening diagnostic tool. However, we report an unusual presentation of Conn's syndrome with normal plasma renin aldosterone ratio.
A 48-year-old man was seen in endocrine clinic with uncontrolled hypertension and severe hypokalemia. Primary hyperaldosteronism was suspected with blood pressure 170/110 mmHg, serum sodium 145 mmol/l, serum potassium 2.4 mmol/l and metabolic alkalosis with serum bicarbonate 29 mmol/l. Initial plasma renin aldosterone ratio was 290 with aldosterone 320 pmol/l and renin 1.1 nmol/l per h. Subsequently blood pressure was controlled and serum potassium was corrected. Repeated plasma renin aldosterone ratio while on doxazosin and normal potassium was again not consistent with Conn's, having aldosterone 365 pmol/l, renin activity 0.8 nmol/l per h with ratio of 450. MRI adrenal confirmed 1 cm nodule in the right adrenal gland.
Despite normal plasma renin aldosterone ratio, clinical suspicion of Conn's disease led to further investigations. Saline infusion test revealed failure of aldosterone suppression at 225 pmol/l with relatively normal renin aldosterone ratio of 450 post saline infusion.
Subsequently, he underwent adrenal veins sampling which showed a significant gradient of aldosterone to the right adrenal gland. (right adrenal vein aldosterone 21 940 pmol/l, cortisol 1630 nmol/l, ratio 13.5; left adrenal vein aldosterone 445 pmol/l, cortisol 1491, ratio 0.3). A laparoscopic right adrenalectomy was done. Histology confirmed benign adenoma consistent with Conn's syndrome.
Two months after surgery, blood pressure was 110/60 mmHg without antihypertensive, serum electrolytes remained normal, normal 24 h urinary potassium at 85 mmol/day, plasma aldosterone 230 pmol/l, renin activity 2.6 nmol/ 1 per h with aldosterone/renin ratio 88. He was discharged from endocrine clinic.
Conclusion
Primary hyperaldosteronism can be a diagnostic dilemma for clinicians. Further investigations should be considered if there is strong clinical evidence despite normal plasma renin aldosterone ratio.

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P65

Peri-operative α-blockade: efficacy of intravenous phenoxybenzamine vs oral phenoxybenzamine in patients with phaeochromocytoma and paraganglioma

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Introduction
Regimens for pre-operative α and β-blockade for patients with secretory phaeochromocytomas/paragangliomas vary widely between centres. The world-wide lack of availability of intravenous phenoxybenzamine (Goldshield) has removed a useful tool in the management of phaeochromocytoma crisis and has necessitated a change in our institution's routine pre-operative strategy. We compare pre, peri and post-operative surrogate measures of blockade in a cohort of patients receiving iv phenoxybenzamine with an oral regimen.
Methods
Of 41 patients with phaeochromocytoma/paraganglioma seen between 2009 and 2012, 19 patients were included in this retrospective audit. Patients were only included if the same surgeon (RAC) and anaesthetist (MA) were present and were excluded if a transfusion was required. All patients had α blockade with oral phenoxybenzamine for at least 3 weeks prior to surgery. In the immediate 3-day pre-operative period five patients had accelerated oral phenoxybenzamine therapy ± intravenous fluids and 14 patients had intravenous phenoxybenzamine ± intravenous fluid. We assessed intraoperative parameters of α blockade efficacy including requirement for sodium nitroprusside (SNP) and intravenous fluids. We assessed postoperative fluid requirement, use of and response to adrenaline, blood pressure and heart rate variability.
Results
Patients treated with intravenous phenoxybenzamine pre-operatively required less SNP (6.4 vs 12.3 mg) and less intra-operative intravenous fluids (2.9 l vs 5.2 l) compared to patients treated with oral phenoxybenzamine. Mean systolic BP in the 3-day pre-operative period was lower in the i.v. group (123 vs 130 mmHg) and the immediate post-operative systolic BP was higher in the iv group (108 mmHg vs 93 mmHg**).** Data including tumour type and size, catecholamine/metanephrine levels, postural BPs, and dose of α and β-blockade will be presented.
Conclusion
Although a small cohort, the data suggest that patients treated with iv phenoxybenzamine have better pre-operative BP control, require less intraoperative intervention and have less post-operative hypotension than patients treated with oral phenoxybenzamine. We invite other centres to report their experience.

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P66

A very interesting presentation: VIP co-secretion by a phaeochromocytoma

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A 62-year-old lady was assessed following discovery of a retroperitoneal tumour on a CT scan. Fifteen years previously she was diagnosed with irritable bowel syndrome with alternating constipation and diarrhoea, however, within the last 5 years, diarrhoea was the dominant feature with bowel opening every 20 min daily. Investigations for inflammatory bowel disease, hyperthyroidism and coeliac disease were negative. Her anal sphincter was found to be non-functioning and a colostomy was performed privately to improve her symptoms, but large volumes of stool continued to be passed daily. An episode of right upper quadrant abdominal pain led to imaging and subsequent referral. She had never been aware of any symptoms such as skin rashes or hypoglycaemia, and her weight was stable. Two years previously she had experienced episodes of palpitations and sweating, although these had resolved and appeared to coincide with menopause. There was no significant family history and clinically, she was not hypertensive. CT demonstrated a 7 cm multi-cystic retroperitoneal tumour adjacent to the tail of the pancreas but uncertainty about pancreatic or adrenal origin. Octreotide uptake was mildly avid. Laboratory investigations showed a markedly elevated vasoactive intestinal peptide (VIP) at 100 pmol/l and urinary dopamine (12 768 mmol/24 h) with normal urinary adrenaline and noradrenaline. Plasma metanephrines confirmed elevated normetanephrine (18 562 pmol/l)
Given these findings, phenoxybenzamine was commenced to optimise blood pressure and a left adrenalectomy was performed. Histology confirmed complete excision and immunohistochemistry was consistent with a phaeochromocytoma. VIP immunoassay was inconclusive due to high background staining, however, a fasting gut hormone profile post operatively confirmed complete normalisation of VIP levels (6 pmol/l) as were plasma normetanephrine (532 pmol/l), suggesting hormone co-secretion. Furthermore, stoma output slowed considerably to normal volumes. We present an unusual case with complex biochemistry that highlights the importance of paying heed to the patient history.

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P67

A single pathology specialty service for hyperthyroid patients improves care and outcomes compared to general endocrine clinics: results and implications of an audit: re-audit cycle of clinical outcomes for differing hyperthyroid care models

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Prior to May 2011, we ran four separate consultant-led endocrine services with six-eight new patient referrals identifiable per week with hyperthyroidism. These were seen 'ad hoc' in general endocrine clinics, where their needs were not prioritised compared to other endocrine referrals, resulting in concern around the timeliness of their care.
We identified 203 patients under active follow-up (FU) (active = on anti-thyroid medications or within 6 months of radioactive iodine). The first audit cycle was carried out to determine care received by patients with thyrotoxicosis in the general endocrine clinics and the time interval between outpatient clinic appointments (OPAs).
Using the July 2006 UK guidelines for use of TFTs, our standards were:
i) All patients with abnormal thyroid function under treatment should be seen within 6 weeks (±7 days)
ii) All euthyroid patients under treatment should be seen within 3 months (± 7 days).
Intervention: Following this review of the 'ad hoc' service, we introduced a dedicated 'weekly multi-disciplinary clinic' hyperthyroid service to streamline care and allow for appropriate follow-up as well as opportunity for liaison with other specialists in those patients who require it.
Results
Standard 1
66% of thyrotoxic/hypothyroid patients were seen within target compared to 32% prior to the new service. Mean FU was 7 weeks compared to 20 weeks.
Standard 2
100% of all euthyroid patients were seen within target compared to 56% prior to the new service. Mean FU was 8 weeks compared to 30 weeks.
More patients achieved euthyroidism in the new hyperthyroid service at both 2nd and 3rd visits.
Conclusion
These data highlight that dedicated hyperthyroid services led to improvements in care quality and outcomes for patients, as well as better use of services and resources. More patients were seen within specified target standards with euthyroid status achieved earlier. There was a significant reduction in re-appointment and non-attendance rates.

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P68

Novel use of subcutaneous octreotide via an insulin pump for postural orthostatic tachycardia syndrome

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Postural orthostatic tachycardia syndrome (PoTS) reflects a disturbance of autonomic function leading to a myriad of clinical features. Subcutaneous octreotide injections and intramuscular long acting preparations of octreotide have been used in the treatment of PoTS. However, inconvenience of frequent injections, side effects and theoretical overexposure, as well as cost of the intramuscular preparation, makes them far from ideal.
We present a case of uncontrolled severe PoTS rectified through the novel use of octreotide via an insulin pump.
A 22-year-old woman presented in April 2002 with episodic palpitations associated with chest discomfort, dyspnoea, and pre-syncope. After 3 years of inconclusive investigations, deteriorating symptomatic control leaving her off work, wheelchair bound, recovering from multiple syncopal related long bone fractures, depression and with a poor quality of life, a diagnosis of PoTS was considered. Diagnostic tilt table testing identified pre-syncope associated with sinus tachycardia, maximum heart rate rise of 42 bpm, and decreases in blood pressure confirming PoTS.
Several therapies including fludrocortisone, slow sodium, midodrine, ephedrine and ivabradine were either ineffective or caused intolerable side effects. In May 2010, SC octreotide, 50 μg every 90 min six times a day (300 μg/24 h), was trialled. Despite her PoTS symptoms improving, she reported excruciating abdominal cramps and diarrhoea following each injection. The severity and frequency of side effects prompted the use of subcutaneous octreotide via an Animas insulin pump in 2011. The patient received 10 μg/h of octreotide for 12 h (120 μg/24 h). The patient reported significant improvements in physical function and ability to maintain upright posture with no side effects. She returned to work and her quality of life improved.
Conclusion
Delivery of octreotide via an insulin pump provides a novel therapeutic strategy in the management of PoTS using lower doses and achieving symptom control with fewer side effects compared to subcutaneous octreotide.

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P69

Management of primary hyperparathyroidism during pregnancy: a case series of the lessons learnt

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Primary hyperparathyroidism (PHPT) is a common condition, affecting approximately 1% of the general population. In women of childbearing age, the correct diagnosis and management is particularly important since PHPT is associated with miscarriage, pre-eclampsia, intrauterine growth restriction, preterm delivery and *postpartum* neonatal hypocalcaemia. We describe a case series of six women diagnosed with PHPT and their management during pregnancy. In four cases, the PHPT was secondary to a parathyroid adenoma whilst in two patients, parathyroid hyperplasia was diagnosed. Four patients underwent parathyroidectomy during the second trimester of pregnancy. One patient was diagnosed during her third trimester and was managed conservatively until delivery. On further follow up, she declined surgery, her calcium levels returned to normal despite raised PTH and she remained asymptomatic. Another patient unfortunately miscarried during her first trimester and underwent a parathyroidectomy thereafter. We discuss the various diagnostic challenges including interpretation of urine calcium to creatinine clearance ratio during pregnancy, the importance of vitamin D replacement during diagnostic work up and the pros and cons of performing surgery during the 2nd trimester of pregnancy. Importantly, in three of these women, hypercalcaemia had been detected prior to conception. This highlights the importance of prompt diagnosis and if PHPT confirmed, appropriate counselling about maternal and fetal risks and the need to expedite surgery. These cases illustrate the difficulties faced in diagnosing and managing women with PHPT during pregnancy, who require close monitoring and definitive management of PHPT. In addition, fetal calcium monitoring immediately *postpartum* is advocated. In some cases it may also be appropriate to undertake genetic testing for multiple endocrine neoplasia, given the age of onset and presentation.

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P70

Normal plasma and urine catecholamines in a patient with symptoms and radiological findings of a phaeochromocytoma cured by laparoscopic adrenalectomy

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A 60-year-old woman was referred with a 14 mm right adrenal mass on a contrast CT abdomen whilst being investigated for left iliac fossa pain and increased bowel frequency. She reported a 2-year history of anxiety attacks, poor sleep, excessive sweating and weight loss. She had hypertension, asthma and recurrent vasovagal syncope and had previously undergone an open cholecystectomy. Her medications included Lansoprazole, Salbutamol, Losartan, Citalopram and Diltiazem. Systemic and abdominal examination was unremarkable. Her blood pressure was normal.
Investigations showed normal FBC, U&Es, LFTs, TSH and glucose. Further biochemical tests showed normal renin and aldosterone levels, DHEAS, and 1 mg overnight dexamethasone suppression test (cortisol 12 nmol/l). She had four 24 h urinary measurements for catecholamines and metanephrines all of which were

normal. Plasma metanephrines were mildly elevated (735 pmol/l; normal range: 80–510) on Citalopram and Diltiazem but repeat measurements following discontinuation of medications were normal (133 pmol/l).
A further non-contrast CT and MRI of the abdomen showed a low attenuation lesion and high signal on T2 weighted images respectively in the right adrenal nodule. MIBG showed significantly elevated tracer activity in the right adrenal nodule.
The patient was counselled about the uncertainty of the diagnosis, risks of surgery and the likelihood that her symptoms may not be cured. After preoperative preparation with maximally tolerated doses of phenoxybenzamine, she underwent retroperitoneoscopic right adrenalectomy. During the procedure, there was clear evidence of blood pressure lability (fluctuations) during tumour manipulation, consistent with a phaeochromocytoma. Her recovery was uneventful. Histology confirmed phaeochromocytoma. Her symptoms resolved and there was a significant drop in plasma metanephrines post-operatively (<40 pmol/l).
This is a rare case of undetectable catecholamine and metabolites in a symptomatic patient with radiological and histological evidence of a small phaeochromocytoma and highlights the importance of symptoms and imaging in the diagnosis of phaeochromocytoma.

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P71

Bariatric surgery in a patient with melanocortin 4 receptor mutation

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Whilst bariatric surgery is the most effective therapy for idiopathic morbid obesity in adults, little is known about its effectiveness in patients with monogenic obesity syndromes. We report 5-year outcome of gastric bypass surgery in a young man with severe super-obesity associated with melanocortin four receptor (MC4R) mutation.
A 22-year-old male with a weight of 221.6 kg and BMI 76.7 kg/m^2 was referred to our centre for bariatric surgery. Previous attempts at lifestyle measures, dieting and treatment with orlistat had been ineffective. His biochemical and haematological profiles and sleep studies were normal. Following multi-disciplinary assessment he underwent Roux-en-Y gastric bypass (RYGB) surgery in July 2007 and achieved weight loss of 60 kg in the first postoperative year. He was treated with sibutramine 15 mg daily from 2008 to 2010 to facilitate further weight reduction. He continues to report good post-surgical appetite suppression and to-date has achieved weight reduction of 75.8% of excess weight.

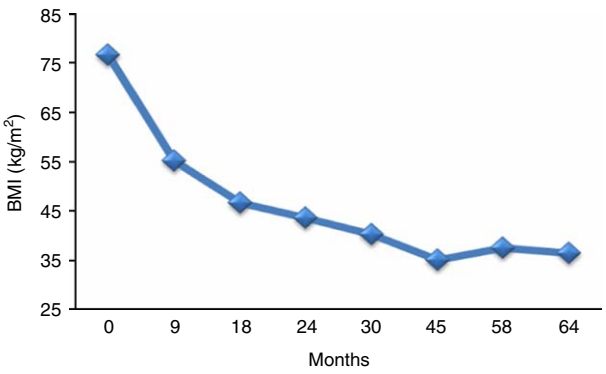

Figure Reduction in body mass index (BMI) following gastric bypass surgery.

Heterozygous MC4R mutations have been associated with dominantly inherited obesity in various ethnic groups and non-surgical interventions are rarely effective in the long-term. One previous report of bariatric surgery in a patient with complete MC4R deficiency reported poor weight loss after gastric banding. We speculate that patients with MC4R mutations achieve superior weight loss outcomes from procedures such as RYGB that produce neurohormonal changes rather than gastric restriction alone.

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P72

Tolvaptan treatment in hyponatraemia due to chronic heart failure

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Hyponatraemia is the commonest electrolyte disorder in hospitals and frequently encountered in patients with heart failure (HF). Elevated circulating levels of arginine vasopressin AVP correlate with disease severity with higher levels in decompensated HF. The activation of AVP from posterior pituitary is mediated through pressure sensitive baroreceptors by impaired cardiac output resulting in increased passive water reasorption in the kidneys with resultant hyponatraemia. This case illustrates decline in sodium level to <120 mmol/l in HF patient despite fluid management and discontinuation of diuretic. A short-term use of Tolvaptan normalises the serum sodium (SNa) and subsequent reintroduction of diuretic without adverse outcome.
Tolvaptan, a V2 receptor antagonist is licensed for treatment of SIADH in Europe based on two RCTs, SALT 1 and SALT 2. In both trials, heart failure with hyponatraemia accounted for 33 and 29% respectively. Tolvaptan significantly increased the average daily AUC for the SNa concentration from baseline to study day 4 through day 30 compared to placebo.
Case: 85-year-old lady with known LVSD with ejection fraction of 50% was admitted with symptoms of heart failure and treated with intravenous diuretic. Though her symptoms improved, her SNa gradually declined despite stopping the diuretic. Biochemistry showed SNa 114 with S osmolality 243 mOsm/kg, Urine osmolality 678 mOsm/kg and urine sodium of <20. She had 'appropriate' ADH elevation due to HF as evidenced by raised UrOsm in the setting of low SOsm. Tolvaptan 15 mg was started and SNa was 131 mmol/l on day 4 with reduction in weight. Tolvaptan was discontinued. Her diuretic was restarted on day 8 and her SNa remained in normal range.
Discussion
Tolvaptan offers additional spectrum in the management of HF to improve symptoms and correction of moderate to severe hyponatraemia in selected cases. (Note: not licensed for HF in Europe)
Declaration of interest
Invited speaker for Otsuka company.

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P73

Hypercalcaemia secondary to colecalcifrol administration in undiagnosed sarcoidosis

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A 32-year-old gentleman, of South-Asian origin was admitted with a 4-week history of abdominal pain, nausea and vomiting. He also had history of polyuria, polydipsia and weight loss over 6 weeks. Just prior to these symptoms he had been started on colecalciferol 20 000 units weekly by his GP for Vitamin D deficiency (25(OH) Vitamin D – 10.3 nmol/l (48–145)). On admission he had adjusted calcium of 4.52 mmol/l and acute kidney injury with his eGFR being 38 ml/min per 1.73 m^2. His bone profile and renal functions were normal earlier, before starting Dekristol. His PTH was suppressed at 0.53 pmol/l (1.1–5.5) and 25(OH) Vitamin D was still low at 37.1 nmol/l. Intravenous fluids and pamidronate resulted in only mild improvement. His chest X-ray showed bihilar lymphadenopathy which prompted us to start him on Prednisolone 30 mg daily. The serum calcium started to improve within 24 hours and the renal functions also improved gradually. His serum ACE level was 229 U/l (8–52) and 1,25(OH)$_2$ Vitamin D was increased at >250 pmol/l (20–120). CT chest confirmed chest X-ray findings and showed multiple cervical lymph nodes. A subsequent lymph node biopsy showed well formed non-caseating granulomata with multinucleated macrophages, confirming the diagnosis of sarcoidosis.
Discussion
The incidence of hypercalcaemia in sarcoidosis is 10–20%. Increased intestinal calcium absorption induced by high serum calcitriol concentrations is the primary abnormality, although a calcitriol-induced increase in bone resorption may also contribute. In sarcoidosis, conversion from calcidiol to calcitriol becomes independent of PTH and occurs in activated mononuclear cells (particularly macrophages). Parathyroid-hormone-related protein may also contribute to the hypercalcaemia in some patients with sarcoidosis. This case illustrates that replacement of oral colecalciferol to treat vitamin D deficiency can precipitate a dangerous elevation in serum calcium levels in previously un-recognised sarcoidosis.

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P74

Spironolactone interference in the immunoassay of androstenedione in a patient with a cortisol-secreting adrenal adenoma

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A 48-year-old man was referred for investigation of uncontrolled hypertension on four agents (olmesartan, felodipine, hydrochlorothiazide and spironolactone) and a 3 cm right-sided adrenal adenoma (pre-contrast Hounsfield units 25). Endocrine investigation for the hypertension and adrenal mass included: androstenedione 19.9 nmol/l 2.8–10.5) (elevated on two occasions on a Siemens Coat-A-Count assay), DHEA 0.7 μmol/l (2.1–15.2), 1 mg overnight dexamethasone suppression test 0900 h cortisol 159 nmol/l and 48 h low dose dexamethasone suppression test 0900 h cortisol 153 nmol/l. ACTH levels were <5 pmol/l. Thus, there was evidence of hormone hypersecretion from two zones of the adrenal gland, and a lipid-poor lesion on imaging; both characteristics would be suspicious for adrenocortical carcinoma.

Spironolactone, diuretics and olmesartan were discontinued to assess his renin-angiotensin-aldosterone system and 6 weeks later a plasma renin activity and aldosterone were performed (3.0 ng/ml per h and 708 pmol/l respectively) and androstenedione was now normal at 3.7 nmol/l (2.8–10.5).

The diagnosis of a cortisol-producing adrenal adenoma was made and the patient underwent laparoscopic adrenalectomy. He was commenced on glucocorticoid cover post-operatively and his 6 week post operative synacthen test revealed glucocorticoid deficiency in keeping with the diagnosis (cortisol 0 min 279 nmol/l, 30 min 366 nmol/l). Histology showed an adrenal adenoma with 0/9 Weiss criteria.

Spironolactone has only recently been described as interfering with the androstenedione assay using the Siemens Coat-A-Count method in three patients with polycystic ovary syndrome. This is the first report of this interference in a patient undergoing endocrine evaluation of an adrenal mass. It is important to recognise spironolactone assay interference; the increased levels of androstenedione as well as cortisol would raise the possibility of adrenal malignancy and influence decision-making when planning the extent of surgical resection.

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P75

An unusual case of confusion and hyponatraemia

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A 73-year-old independent female presented with reduced consciousness following a tonic-clonic seizure. Investigations revealed acute hyponatraemia, with a serum sodium of 103 mmol/l. Cortisol reserve and thyroid function was normal and the biochemical diagnosis was consistent with SiADH. A CT brain scan was normal. Hypertonic saline was commenced with empirical anti-viral and antibiotic therapy. Lumbar puncture revealed a slightly elevated CSF protein and her EEG showed non-specific changes. Her conscious level improved, but there was evidence of ongoing confusion.

Clinical concern of central pontine myelinolysis following correction of the hyponatraemia was excluded by MRI brain scan. She was discharged following functional recovery but re-presented 5 days later with acute worsening of confusion, new paranoid delusions and emotional lability.

Repeat investigations showed no metabolic abnormality. Paraneoplastic and autoimmune encephalitis antibody serology was requested. After 2 weeks, these returned from the Oxford Reference Laboratory showing a positive Voltage-gated potassium-channel (VGKC) complex antibody titre. A diagnosis of autoimmune limbic encephalitis was made and 3 days intravenous methylprednisolone treatment was given. The patient made a full cognitive recovery, allowing discharge home independently.

Conclusion

Endocrinologists are increasingly asked to assess hyponatraemic patients both for diagnostic and management purposes. Confusion is common with hyponatraemia, but should reverse if the metabolic situation is corrected appropriately. This case shows the importance of considering a diagnosis of autoimmune encephalitis once other causes of SiADH and cognitive impairment have been excluded.

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P76

Hypernatraemia with reset osmostat associated with secondary hypogonadism

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We report two cases of hypernatraemia with reset osmostat and pituitary dysfunction.

A 35-year-old male was referred with Graves' thyrotoxicosis associated with hypokalaemic periodic paralysis and an incidental serum sodium 154 mmol/l. He complained of polyuria and nocturia but denied excessive thirst and was otherwise well. Height was 193 cm with BMI 29.5. He had gynaecomastia and sparse body hair. He had a small 6 ml right testicle (originally undescended) and 15 ml left testicle. A very high arched, possibly cleft palate was noted suggesting a midline defect. Serum osmolality was 314 mOsm/kg with urine osmolality 1025mOsm/kg. Testosterone was 5.0 nmol/l, LH 5.6 IU/l and FSH 5.0 IU/l. Prolactin, IGF-1, synacthen test, renin and aldosterone were normal. Pituitary MRI was normal. Urine osmolality dropped in response to a water load although he remained hypernatraemic with hyperosmolar plasma. There was direct evidence of osmoregulatory control over AVP levels consistent with hypernatraemia and reset osmostat.

An 18-year-old male was admitted following an incidental serum sodium 163 mmol/l whilst being investigated for joints pain. He denied excessive thirst or polyuria. Examination showed BMI 40.4, short stature (154 cm), small hands, size 5 feet and gynaecomastia. Testicular volumes were 15ml. Serum osmolality was 330 mOsm/kg, urine osmolality 731 mOsm/kg and urine sodium 172 mmol/l. Initial hospital treatment with 5% Glucose reduced his sodium to 155 mmol/l, urine osmolality to 296 mOsm/kg and urine output increased. Prolactin was 2000 mIU/l, LH 6.3 IU/l, FSH 1.0 IU/l and testosterone 5.7 nmol/l. IGF1 was low with peak GH 4.5 μg/l on ITT. TSH, FT4 and synacthen test were normal. Pituitary MRI was normal. He has been started on dopamine agonist therapy.

'Essential hypernatraemia' is rare and usually secondary to traumatic brain injury or surgery. In our cases, there was no such history, and both patients had partial hypopituitarism. The likely cause in both was congenital and in one case, there was evidence of a congenital midline defect.

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P77

A case of dry beriberi following bariatric surgery

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Introduction

Over 9000 bariatric surgeries are conducted annually in the UK and post-operative micronutrient deficiency is common. We report a case of dry beriberi secondary to thiamine deficiency following Roux-en-Y bypass surgery.

Case presentation

A 45-year-old Caucasian obese lady (155 kg, BMI 57) presented with progressive proximal limb weakness, upper and lower limb paraesthesia, ataxia and athetoid tremors 18 months following Roux-en-Y gastric bypass surgery. Post-operatively, she lost 57 kg and maintained a healthy diet with moderate alcohol intake (<14 units/week). She admitted non-compliance with multivitamin and Adcal D3 supplementation. Examination revealed grade 3/5 weakness in upper and lower limbs with 'glove and stocking' impaired touch and vibration sensation. Deep tendon reflexes were impaired in lower limbs with preserved extrapyramidal and cerebellar functions. Nerve conduction studies revealed mixed sensory and motor conduction defect. Serum calcium, vitamin B12, magnesium, ferritin, copper, phosphate and vitamin D levels were normal. Initial thiamine status determination was unsuccessful due to unavailability of the test locally. Thiamine deficiency was diagnosed clinically and was commenced on regular parenteral thiamine infusions (100 mg/day) and oral thiamine (300 mg/day). Serum thiamine after two thiamine infusions was 170 nmol/l (66–200).

Progress

Over the next 24 months, complete resolution of proximal weakness and ataxia was noted. Tremors, mild paraesthesia and grade 1/5 motor weakness in hand muscles persist despite 3-monthly parenteral thiamine and vitamin B12 therapy, oral thiamine (300 mg/day), vitamin B compound and Adcal D3.

Conclusion

Laboratory assessment of thiamine status is expensive, cumbersome and is not widely available. Normal serum thiamine level does not exclude thiamine deficiency and a high index of clinical suspicion is warranted to diagnose

thiamine deficiency states (dry beriberi, wet beriberi and Wernicke Korsakoff syndrome). Biochemical and clinical monitoring of micronutrient deficiencies should be mandatory for all peri-operative bariatric surgery patients, preferably by adopting a locally agreed protocol.

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P78

Giant parathyroid adenoma extending into the mediastinum: a case report

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Introduction
85% of cases of primary hyperparathyroidism are caused by a parathyroid adenoma. These are usually small in size, in the range of 70 mg to 1 g. Those weighing more than 2 g are classified as 'giant adenomas'. Few adenomas weighing more than 30 g have been described in the literature. Here we present one such case.
Case report
A 52-year-old female presenting with lethargy was found to have a corrected calcium of 3.15 mmol/l with a parathyroid hormone of 122.5 pmol/l. Imaging of the parathyroids was performed. Sestamibi scanning demonstrated a large area of high uptake, not typical of a parathyroid adenoma. Computed tomography revealed a mass, 9 cm in maximum dimension, extending from the inferior pole of the right lobe of the thyroid to the right side of the mediastinum, and into the subcarinal area. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS–TBNA) of this mass showed acinar structures, morphology and phenotype favouring parathyroid adenoma. This was confirmed histologically after surgical removal of a large single parathyroid adenoma, weighing 92.7 g, under the joint care of maxillofacial/cardiothoracic surgical teams. Bisphosphonate treatment was required to control calcium levels (which rose to 3.8 mmol/l) pre-operatively. The patient experienced post-operative hypocalcaemia, requiring calcium infusion, high dose oral calcium supplementation and alphacalcidol treatment.
Discussion
This case is one of the biggest giant parathyroid adenomas described, the largest described weighed 116 g. In general adenoma weight correlates with functional status of the gland, only one case of a giant non-functioning adenoma has been reported. Primary hyperparathyroidism is more common in women but there is relative male overrepresentation in cases of large adenoma. Whilst malignancy does not appear to be more common in cases of giant parathyroid adenoma, such cases may represent a subset of parathyroid lesion with pronounced features of primary hyperparathyroidism and specific genomic aberrations.

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P79

Tertiary hyperparathyroidism due to chronic severe vitamin D deficiency in ethnic minority patients: a case series

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Introduction
Vitamin D deficiency is common in ethnic minority population living in UK. It is a relatively easily treatable condition. However, if left untreated it may lead to chronic secondary hyperparathyroidism and onto tertiary hyperparathyroidism. We report three cases of severe vitamin D deficiency who presented with hypercalcaemia.
Cases
We report three patients. 60 and 46 years old ladies, both of south-asian origin, a 46 years old man of Somalian origin referred to the endocrinology services with severe hypercalcaemia. Referred calcium levels were 3.20, 3.79 and 2.98 mmol/l respectively. Parathormone (PTH) levels were 625, 890 and 639 pg/ml. Vitamin D levels 11, <10 and 7 nmol/l (normal 70–250). PTH levels were 484, 532 and 234 pg/ml respectively after high dose colecalciferol replacement. Calcium levels did not change significantly.
Imaging
Single left ectopic inferior parathyroid adenoma in the first lady on NM parathyroid MIBI scan. Left superior, left inferior and the ectopic left inferior parathyroid glands were removed at surgery. Postoperative calcium was 2.47 mmol/l and PTH was 32 pg/ml.
A right single inferior parathyroid adenoma was identified on the NM Parathyroid MIBI scan in the second lady. A right inferior parathyroid adenoma was removed. Postoperative calcium was 2.44 mmol/l and a parathormone level 33 pg/ml.
The gentleman had a left superior and a right inferior ectopic parathyroid detected on USS and NM parathyroid MIBI scan. He is awaiting surgery.
Discussion
Tertiary hyperparathyroidism (TPHT) has been commonly associated with renal transplant and end stage renal failure patients. Here we present three cases of THPT which we believe were secondary to untreated chronic severe vitamin D deficiency. Vitamin D deficiency is a fairly common condition particularly in the ethnic minority population in the UK, both men and women. Early recognition and treatment of this condition could prevent the progression to THPT and save surgical intervention and thus reducing significant healthcare cost.

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P80

Hypercalcaemia due to simultaneous presentation of primary hyperparathyroidism and metastatic oesophageal cancer

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Introduction
We report a patient with hypercalcaemia secondary to parathyroid hormone related peptide (PTHrp) from metastatic oesophageal cancer and co-existing primary hyperparathyroidism.
Case report
A 52-year-old lady was admitted to the hospital with a 2-week history of right scapula pain, reduced appetite and weight loss.
Blood test revealed an adjusted calcium of 3.99 nmol/l (NR 2.1–2.55 nmol/l), PTH of 147 ng/l (NR 15–65 ng/l), PTHrp of 4.3 pmol/l (NR 0.0–1.8 pmol/l) and normal myeloma screen. Normal 25-OH vitamin D levels and renal functions excluded the possible secondary PTH elevation.
CT scan showed evidence of lung and liver metastasis but the site of primary carcinoma could not be identified. Bone scan did not reveal any bony lesion. Liver biopsy confirmed metastatic carcinoma with possible lung or gastrointestinal tract (GI) as a primary site. She underwent an upper GI endoscopy which confirmed squamous cell cancer on oesophageal biopsy.
Hypercalcaemia initially responded well to intravenous fluid and bisphosphonate therapy and she was discharged home after noticeable clinical and biochemical improvement. She was readmitted with symptomatic hypercalcaemia resistant to further fluids and bisphosphonates. A trial of chemotherapy did not improve her hypercalcaemia and 2 months after her initial diagnosis, she passed away.
Conclusion
Hypercalcaemia is a common occurrence in malignancy and usually confer a poor prognosis. In our patient, hypercalcaemia was secondary to both metastatic oesophageal cancer and primary hyperparathyroidism. Primary hyperparathyroidism in this setting is mere an interesting observation and does not influence the course of patients' treatment or outcome.
There have been case reports linking an association between metastatic breast cancer and primary hyperparathyroidism and their simultaneous presentation, but none have yet reported with metastatic oesophageal cancer.

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P81

'There is nothing more deceptive than an obvious fact', Sherlock Holmes: a case report of thyroid sarcoidosis

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A 53-year-old caucasian female presented with tremor, palpitations, sweating, breathlessness and chest discomfort. Examination revealed a non-tender, large diffuse goitre and TFT showed an elevated T_4 and undetectable TSH. Thyroid antibodies were elevated; 115 IU/ml (NR <35) and thyroid ultrasound confirmed diffuse vascular goitre. Patient was diagnosed with thyrotoxicosis with a likely aetiology of Grave's disease.
Carbimazole was commenced which normalised the thyroid function, but was stopped when the patient developed agranulocytosis. Neutrophil count recovered, however symptoms returned with the addition of thyroid eye disease and deranged liver function (contraindicating treatment with propythiourocil). Abdominal ultrasound revealed no hepatobiliary tract abnormality. Virology, immunoglobulins, ferritin, PT, autoantibody and infection screens were negative.

Echocardiogram demonstrated moderate tricuspid regurgitation, dilated right atrium and elevated pulmonary artery pressure. CXR showed bulky hilar, reported as not clinically significant. The patient was pre-treated with B-blockers and lugol's iodine. She underwent uncomplicated total thyroidectomy once TFT's had normalised. Histology showed diffuse hyperplasia, areas of fibrosis and several non-caseating granulomas. Post-operatively, liver function normalised spontaneously. Despite normal thyroid function and thyroxine replacement, breathlessness persisted. Serum ACE level was elevated at 114 U/l (NR11–55). Ultimately, the patient was referred to the respiratory team for management of pulmonary sarcoidosis.

This case raises the following points: A rare but well recognised case of thyroid gland sarcoidosis coexisting with Grave's disease. Liver dysfunction can be explained by thyrotoxicosis, thionamide treatment and sarcoidosis. Although the symptoms aligned with Grave's hyperthyroidism, this was not the sole cause of the patient's symptoms, particularly the breathlessness. Post-thyroidectomy, patient remained breathless and disabled. The initial hilar adenopathy was discounted, however was one of the missing pieces of the puzzle in the underlying diagnosis. Lesson; even though a symptom appears to be explained by one diagnosis there may be more than one condition contributing to symptom complex!

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P82

A case of severe hypoaldosteronism following unilateral adrenalectomy for Conn's syndrome

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A 58-year-old female presented with a 20-year history of resistant hypertension and hypokalaemia, with normal renal function. Investigations confirmed primary hyperaldosteronism that was not suppressed following a standard saline infusion test. CT scanning revealed a right-sided adrenal mass of 1.3 cm, with a signal intensity of −1 HU. The left adrenal was normal in appearance. A LDDST excluded ACTH-independent Cushing's syndrome. Adrenal vein sampling confirmed right-sided unilateral hypersecretion of aldosterone; she underwent an uneventful right adrenalectomy in October 2011, following which her BP was controlled on atenolol alone.

In November 2011 she presented to other hospitals with acute kidney injury, postural hypotension and hyperkalaemia. In July 2012 she was admitted to our unit and was found to have a normal anion-gap metabolic acidosis, consistent with type IV renal tubular acidosis. A SST confirmed a normal adrenal–glucocorticoid axis (peak cortisol 630 nmol/l). Her aldosterone level was inappropriately low, given hypovolaemia and hyperkalaemia, suggesting hypoaldosteronism (plasma aldosterone 100 pmol/l; plasma renin activity 4.4 pmol/ml per h). In keeping with this, her biochemical abnormalities normalized following mineralocorticoid replacement. An attempt to reduce the dose of fludrocortisone resulted in a further reversible recurrence of both hyperkalaemia and acute kidney injury. She is now taking 100 µg fludrocortisone TDS, and remains well. Her atenolol has been changed to amlodipine, as ß-blockade may have prevented adequate recovery of her renin-aldosterone axis.

Post-operative hypoaldosteronism is well documented in cases of unilateral adrenalectomy for aldosterone-producing adenomas. This may relate to a decrease in adrenal mass, or a transient suppression of the contralateral gland (1). However, it is rare for this to be prolonged, or severe. This may occur in up to 5% of cases, and responds to mineralocorticoid treatment (2). Our case illustrates the importance of follow-up in the post-operative period.

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P83

SDHB mutation and a large asymptomatic paraganglioma in a young woman: the importance of taking a good family history

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Succinate dehydrogenase subunit B (SDHB) mutations are associated with a high risk of developing pheochromocytomas, paragangliomas and renal cell tumours. The risk of malignancy is also higher than that of other SDH mutations.

A 23-year-old woman was referred to endocrine clinic following confirmation of an SDHB mutation. Her family was screened when a relative underwent a medical, prior to starting a new job, and a significant family history of renal tumours was discovered. The patient's grandmother had two siblings with renal cancer and a further sibling with bone metastases of unknown origin. The patient's uncle, mother and brother were found to have SDHB mutations. The uncle was discovered to have a renal tumour whereas the mother had normal imaging and biochemistry; the brother is currently under investigation.

The patient had no symptoms of a catecholamine-producing tumour, did not complain of back pain or abdominal pain and had normal urinary catecholamines. A routine screening CT scan of the abdomen, however, revealed a large, heretogeneously enhancing mass in the right para-aortic region, arising from the organ of Zuckerkandl. Pre-operative clinical examination confirmed the presence of an abdominal mass. Surgical resection of the mass was difficult as it was adherent to all surrounding tissue. The mass measured 10×6.3×11.2 cm and immunohistochemistry confirmed it to be a benign paraganglioma.

This case demonstrates the importance of genetic screening when considering SDH mutations. Due to non-penetrance, a three-generation family history is necessary as the relevant diseases may appear to 'skip' a generation. This young woman had a very large tumour which was detected in the absence of symptoms and would not have been diagnosed if the physician conducting the medical had missed the strong family history of renal cancer. Early detection is especially important due to the malignant potential of these tumours.

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P84

The challenges of a dopamine secreting paraganglioma

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A 39-year-old female presented with an 18-month history of borderline hypertension, headaches, palpitations and some anxiety symptoms. Both parents also had hypertension. Blood pressure was 160/102 on no medications. She had a large single cafe au lait spot but no neurofibromata. Urinary dopamine levels were repeatedly elevated (5398–8653 nmol/24 h ($n<3900$)) with normal noradrenaline and adrenaline levels. Serum calcium was also elevated at 2.83 mmol/l ($n=2.2–2.6$). Meta-iodobenzylguanidine (MIBG I-123) scanning indicated a single focus of activity further defined on SPECT–CT as a 12 mm mass in the lower para-aortic region. Parathormone was elevated at 274 pg/ml ($n=15–70$) and a nuclear uptake scan indicated a right lower parathyroid focus. Calcitonin was unmeasurable. Following control of blood pressure with amlodipine and lisinopril she proceeded to surgery where a black lobulated paraganglioma was removed at the organ of Zuckerkandl. A 30 s asystolic episode occurred on first manipulation of the tumour. Urinary dopamine levels normalised, antihypertensives were reduced and 5 months later a parathyroid adenoma was removed uneventfully. Antihypertensives have recently been withdrawn. Genetic testing to date has been negative including MEN2a, succinate dehydrogenase B, C and D mutations. Dopamine secreting paragangliomas are extremely rare and are usually metastatic at diagnosis. They are usually associated with nonspecific symptoms, normotension and present with mass effects. Alpha blockade is contraindicated because of its association with cardiovascular collapse which is due to the unopposed hypotensive action of dopamine when the pressor catecholamines are blocked. This is the first reported case presenting with a coexistent parathyroid adenoma.

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P85

Lithium-induced hyperparathyroidism successfully treated with cinacalcet: two case reports

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Background

Lithium-associated hyperparathyroidism (HPT) is an under-recognised side effect of chronic lithium therapy. Cessation of lithium may precipitate relapse of psychiatric illness. Potential treatment with cinacalcet has been described in two case series recently.

Aim
We present two cases of HPT secondary to lithium therapy used for bipolar affective disorder, which were successfully managed with cinacalcet.
Results
Patient 1 is a 64-year-old gentleman with a history of lethargy, nocturia and polyuria who was found to have hypercalcaemia and HPT. Sestamibi/SPECT scans did not detect any parathyroid adenomas but surgical exploration revealed an enlarged right superior parathyroid gland which was excised. Histopathology confirmed a parathyroid adenoma. However, the calcium and parathyroid hormone (PTH) remained consistently elevated after parathyroidectomy and cinacalcet was introduced at a dose of 30 mg once daily. Calcium and PTH levels improved over 15 months. Patient 2 is a 60-year-old gentleman who was investigated for tiredness, polyuria and pathological fractures and found to have hypercalcaemia and HPT as well as nephrocalcinosis and osteoporosis. No definite parathyroid adenomas were identified on Sestamibi/SPECT scans and a trial of cinacalcet (30 mg once daily titrated with biochemical response) was started. This resulted in normalisation of both calcium and PTH levels within 2 years. In both cases, cinacalcet was well tolerated and the patients improved symptomatically with no further complications from their underlying conditions.

Table 1

		Before cinacalcet	On cinacalcet[a]	Reference range
Patient 1	Calcium (adj.)	3.02	2.65	2.20–2.60 (mmol/l)
	PTH	43.0	22.2	1.5–7.6 (pmol/l)
Patient 2	Calcium (adj.)	2.64	2.54	
	PTH	22.5	7.0	

[a]Most recent results on cinacalcet therapy.

Conclusion
Cinacalcet is known to be effective in primary hyperparathyroidism but our observations also support the use of this calcimimetic agent in lithium-induced hyperparathyroidism as a potential alternative to surgery.

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P86

Recurrence of a brown tumour in a patient with secondary hyperparathyroidism due to severe vitamin D deficiency

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In May 2011 a 55-year-old South East Asian man had a tumour removed from the floor of his mouth. The histology was consistent with a brown tumour of hyperparathyroidism. His PTH was raised at 12.3 pmol/l (NR 1.6–7.5) and he was referred to the endocrinology clinic. His serum calcium was 2.22 mmol/l (NR 2.2–2.6 mmol/l) and his serum 25 (OH) vitamin D level was <15 (NR >50 nmol/l) with a normal alkaline phosphatase. He had no symptoms of osteomalacia. He is a vegetarian. His past medical history included hypertension, hyperlipidaemia and a pulmonary embolism in 2005. His results were consistent with secondary hyperparathyroidism due to severe vitamin D deficiency. He was given replacement therapy and when discharged from the clinic in November 2011 his PTH was normal at 3.8 pmol/l, his serum calcium was 2.35 mmo/l and his vitamin D level was 165 nmol/l. He was advised to take 25 µg (1000 IU) of colecalciferol lifelong. In March/April 2012 the patient noticed swelling in the right maxilla which progressively got bigger. A CT scan of the lesion showed an expansile lesion in the right maxillary alveolus. Biopsy again showed features consistent with a brown tumour of hyperparathyroidism. His repeat PTH was normal at 3.9 pmol/l with a normal serum calcium of 2.34 mmol/l and his serum 25 (OH) vitamin D level was 48 nmol/l. His urinary calcium excretion was normal (5.9 mmol/24 h – NR 2.5–7.5). He was advised to increase his colecalciferol to 50 µg (2000 IU) per day. An ultrasound scan of his parathyroid glands was essentially normal.
Brown tumours are non-neoplastic lesions resulting from abnormal bone metabolism and are known to be associated with primary hyperparathyroidism. There have been case reports of peripheral brown tumour in the maxilla as manifestation of secondary hyperparathyroidism associated with chronic renal failure but to our knowledge there have been no reports of a brown tumour due to secondary hyperparathyroidism as a result of severe vitamin D deficiency. Furthermore it is unclear why the patient developed a further brown tumour after his secondary hyperparathyroidism had been corrected.

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P87

An uncommon presentation of a common endocrine condition

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A 55-year female from low economic background was referred from the local dental hospital where she had presented with a swelling near her left lower 3rd molar which caused her significant difficulty in eating. On further questioning she conceded to having progressive difficulty in walking and bony pains over the past 6 months with increasing dependency for daily activities. She was frail with proximal muscle weakness and widespread bony tenderness but no other significant neurological abnormalities. She also had two firm non-tender swellings over the lower half of her right forearm and over the right hand. Total calcium 2.3 mmol/l (2.2–2.6), phosphorus 0.63 mmol/l (0.81–1.4), albumin 33 g/l (35–50), alkaline phosphatase 124 U/l (42–98), parathyroid hormone 2842 ng/l (8–51), Serum creatinine 61.9 µmol/l (44–100) and vitamin-D 14.6 ng/ml (30–100). Skeletal X-rays showed radiolucent oval lesions over the lower 1/3rd of radius and the 4th metacarpal base with appearances suggestive of Brown tumours. This diagnosis was confirmed by biopsy of the oral lesion with the overall picture suggestive of long-standing vitamin-D deficiency and secondary hyperparathyroidism. She was commenced on 60 000 units of cholecalciferol weekly. By 6 weeks there was a significant improvement in mobility, bony pains and sense of well-being. At 3 months despite continued overall improvement she re-consulted oral surgeons to request surgical excision of the persistently symptomatic oral lesion. She is awaiting this procedure and biochemical re-assessment.
A Brown tumour is recognised sequelae of long-standing primary hyperparathyroidism but the presence of multiple tumours has not been reported in a patient with vitamin-D deficiency. The persistence of symptomatic oral tumour requiring excision is also unusual.
In conclusion, long standing vitamin D deficiency presents in a variety of ways and a high index of suspicion needs to be maintained in high-risk populations.

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P88

Rare case of pheochromocytoma presenting in pregnancy

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Introduction
We submit a rare presentation of pheaochromocytoma in pregnanacy, diagnosed just before delivery, posing complex management difficulties.
Case presentation
A 24-year-old primipara had headache, hypertension and visual disturbance but without any palpitations or diaphoresis. Her headache was persistent during pregnancy. Investigations at 39 weeks of gestation showed normal plasma Normetadrenaline at 885.0 (120–1180) but raised Metadrenaline raised at 1849 (80–510) leading onto a diagnosis of pheochromocytoma. There was an initial diagnostic dilemma, if the raised catacholamines are due to stress with pregnanacy. However ultrasound abdomen showed left adrenal enlargement. She was started on phenoxybenzamine and the blood pressure was controlled. Labour was successfully managed with adequate α blockade and a healthy baby was delivered by Caessarean section at 40 weeks of gestation. After delivery she was started on Bisoprolol along with increasing dose of phenoxybenzamine. Left adrenal pheochromocytoma was confirmed by CT Abdomen showing a 9×6 mm left adrenal adenoma which was confirmed by MIBG Scan. Laparoscopic left adrenalectomy was planned with controlled α and β blockade.
Discussion
Hypertension is a common problem in pregnancy that can result in significant maternal and foetal morbidity and mortality. The common causes include pre - eclampsia, gestational hypertension and essential hypertension. Although pheochromocytoma is a rare cause of hypertension in pregnancy (0.007% of all pregnancies), it can lead to potentially life-threatening cardiovascular complications for the mother and increased foetal mortality if left undiagnosed and untreated. In pregnancy, depending on the gestation at which diagnosis is made, the optimal timing for surgery is during the late first or early second trimester. When the pregnancy is more advanced, medical management followed by combined caesarean section and tumour resection closer to term is preferred.

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P89

Non islet cell tumour hypoglycaemia resistant to medical treatment

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A 73 years old gentleman with a diagnosis of mesothelioma presented with symptoms typical of hypoglycaemia. Other than the expected abnormal chest signs there were no significant examination findings.
Capillary glucose was unrecordable; lab testing confirmed serum glucose of 0.9 mmol/l. He had no history of diabetes mellitus or any medication that may induce hypoglycaemia. There was a slight rise in CRP and white cell count was elevated. There was no clinical evidence of infection. Routine biochemical tests were normal. Chest X-ray showed extensive left chest shadowing.
Intramuscular glucagon and intravenous dextrose initially improved blood glucose but episodic symptomatic hypoglycaemia continued. Blood glucoses fluctuated between 0.9 and 2 mmol/l. Despite aggressive treatment with dextrose 10% and additional dextrose 50% boluses he continued to have frequent hypoglycaemia.
Having previous treatment with steroids a short synacthen test was requested, which was normal. A recent CT scan showed no adrenal, hepatic or pancreatic lesions.
With no other apparent cause a presumptive diagnosis of non islet cell tumour hypoglycaemia was made. This was confirmed by suppressed insulin (<10 pmol/l) and c-peptide (<94 pmol/l) levels with a high IGF2:IGF1 ratio (19.3). Chest ultrasound confirmed significant mesothelial tumour with little pleural fluid. He was treated with high dose corticosteroid, diazoxide and octreotide infusion. Severe hypoglycaemia remained problematic necessitating continued glucose infusions. He was unfit for chemotherapy or debulking surgery and deteriorated rapidly before dying 7 days after admission.
Non islet cell tumour hypoglycaemia is a rare paraneoplastic phenomenon. Treatment involves reduction in tumour size by chemotherapy or surgery. Hypoglycaemia can remain significant and resistant to medical management strategies.

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P90

An unusual case of pancreatitis: a case report

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A previously fit and well 49-year-old gentleman presented to hospital with vomiting and myalgia. Three weeks previously he had been admitted with acute pancreatitis, but no cause was found. An incidental hypercalcaemia was also noted at that time. He had lost approximately two stone in weight in the previous three months and had become more short of breath on exertion. He had a strong family history for type I diabetes mellitus, but otherwise there was no significant history.
On this admission he was found to have a high corrected calcium of 3.14, with a low parathyroid hormone (<0.3). Further tests revealed that he had a low vitamin D, a normal myeloma screen, PSA and serum ACE. Imaging performed was unremarkable, with a normal chest X-ray, CT thorax and NM whole body bone scan. He was treated with IV fluid resuscitation and pamidronate.
Additional investigations came back and exposed that he was thyrotoxic with $T_4 > 75$ and supressed TSH <0.03. He had a positive TSH receptor antibody of 6.2. A NM thyroid scan with uptake technetium showed enlargement and marked increased uptake in both thyroid lobes consistent with Grave's disease. He was commenced on carbimazole and monitored in an outpatient endocrinology clinic with a normal follow up calcium level of 2.20.
Hypercalcaemia is most commonly caused by primary hyperparathyroidism[1]. Malignancy is another common cause and together they account for the majority of cases of hypercalcaemia (1).
Thyrotoxicosis has been found as a sole cause of hypercalcaemia, however, significant symptomatic hypercalcaemia is rare (2). There have been only a few other case reports of similar symptomatic hypercalcaemia with hyperthyroidism (3). However, in this case his thyrotoxicosis also lead to his pancreatitis through having hypercalcaemia. This case highlights that thyroid function tests should always be done when presented with a high calcium level.
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P91

A painful neck in a young well looking man presenting to A&E

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A 38-year-old man presented on the acute medical take with a week's history of sore throat, dysphagia, neck swelling, and fever. One month prior he had suffered a respiratory tract infection, which resolved without antibiotics. He had no notable past medical and family history. He was a non-smoker and drank 15 units of alcohol per week. On examination he looked well, was afebrile, sweaty and flushed; chest was clear, heart sounds were normal, regular pulse (110 bpm) and BP 148/88 mmHg. His anterior neck was erythematous, his right thyroid lobe was smoothly enlarged with no bruits heard over his thyroid. There was no palpable cervical lymphadenopathy and no evidence of tonsillitis. An initial clinical diagnosis of de Quervain's thyroiditis was made. Blood tests revealed WCC 20, CRP 515 and normal thyroid function. Due to the neck erythema and high CRP an ultrasound was requested.
Ultrasound revealed a very large complex pus collection extending to the right sternocleidomastoid muscle. Samples were obtained but percutaneous drainage was not possible. HIV test was negative, Group A beta haemolytic streptococcus was cultured from the pus but blood cultures were sterile. Empirical intravenous co-amoxiclav was changed to intravenous ceftriaxone and oral clindamycin. He was discharged after 5 days and continued outpatient treatment. After 10 days of ceftriaxone, 20 days of clindamycin and 2 weeks of co-amoxiclav with additional amoxicillin, there was complete radiological resolution of the abscess. Video fluoroscopy and direct phyarngoscopy excluded a piriform sinus fistula. All blood tests normalised and no mycobacteria were cultured from extended culture of the abscess fluid.
Viral thyroiditis remains the most likely diagnosis in a patient with a painful thyroid, but thyroid abscess must be considered as although it is rare, its management is different from viral thyroiditis and it is associated with a mortality of up to 12%.

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P92

Life threatening airway obstruction secondary to a large probable parathyroid cyst

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Parathyroid cysts are rare, usually asymptomatic and typically present as a neck lump. They are most commonly detected in middle-aged women and can occasionally present with compressive symptoms. True parathyroid cysts are non-functional and benign in nature, allowing a more conservative approach to their management in many patients. We present the case of an 82-year-old lady with a past medical history of a presumed parathyroid cyst which was drained by the ENT surgeons. Three years later, she presented to hospital with sudden onset shortness of breath, throat pain and three months of increasing dysphagia. Examination and initial investigations were consistent with upper airway obstruction and a chest radiograph showed a large retrosternal mass. Computed tomography revealed a large 8×5 cm cyst causing tracheal compression and deviation. The patient underwent drainage of the cyst via ultrasound and fluoroscopic guidance with full resolution of symptoms. Histology and intra-cystic PTH levels were consistent with a large parathyroid cyst however an alternative diagnosis of a 3rd branchial arch cyst could not be excluded. We discuss the prevalence and presentation of such cysts and different management options. This case illustrates a rare, yet important cause of a neck lump that can present with life-threatening upper airway obstruction.

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P93

Tuberous sclerosis: an uncommon cause of hyperprolactinemia

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Objective
To report a case of tuberous sclerosis presenting with hyperprolactinemia.
Methods
Clinical, laboratory and radiographic data are reported on a 26-year-old female presenting with galactorrhea and menstrual irregularities.
Case report
A 26-year-old female with no premorbidities presented with complaints of galactorrhea for the past 10 days and menstrual irregularities over the past six months. Galactorrhea was spontaneous. Her last childbirth four years ago was uneventful. She had no head ache, vomiting, and visual impairment. She denied any history of substance abuse, drug intake hypothyroidism, chronic liver or kidney diseases, and epilepsy. She was a well nourished female with mild pallor, tiny nodule on face, subungual fibroma in hands. There was spontaneous galactorrhea and mildly tender breasts without any signs of inflammation. Systemic examination was entirely normal with normal IQ. Ophthalmologic evaluation revealed white disk shaped retinal hamartoma. Routine laboratory investigations including renal and liver function tests, thyroid profile were normal. Serum prolactin was 85 ng/ml with FSH −4.66 and LH −4.21 mIU/ml. Tests for evaluation of other anterior pituitary hormones were normal. Abdominal and pelvic ultrasound revealed no abnormality. Chest X ray showed bilateral interstitial infiltrates. Echocardiogram of heart was normal. Computed tomography (CT) scan revealed multiple intracerebral calcifications. These calcified lesions/ subependymal hamartomas are seen along the lateral surface of the lateral ventricles giving rise to characteristic candle dripping appearance. Magnetic resonance imaging (MRI) of the brain ruled out the presence of any pituitary mass. The combined clinical scenario along with the radiologic findings leads to the diagnosis of TSC with hyperprolactinemia. Patient was prescribed cabergoline 0.5 mg twice daily which resulted in amelioration of galactorrhea and regularization of menses.
Discussion
Tuberous sclerosis (TSC) is a multi system genetic disorder which infrequently affects the endocrine system. Cushing's disease, hypoglycemia secondary to insulinomas, precocious puberty, thyrotoxicosis, hypercalcemia secondary to parathyroid adenomas, hyperprolactinemia and acromegaly have all been reported in TSC patients. The circulating prolactin of our patient may be of pituitary origin or may possibly be secreted ectopically by a hamartoma.
Conclusion
TSC patients develop hormone secreting tumors involving the neuroendocrine system.

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P94

Acute diabetic autonomic neuropathy as phaeochromocytoma mimic

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A 20-year-old man with a 5-year history of poorly controlled type 1 diabetes presented with epigastric pain, bloating and weight loss. He had attended DAFNE recently and had been commenced on an insulin Pump resulting in improvement of HbA1C from 114 to 76 mmol/mol over a 4-month period. Blood pressure was elevated (157/108 mmHg) with a resting tachycardia of 110. Haemoglobin was elevated at 18.7 g/dl. 24 h blood pressure monitoring revealed an average diastolic blood pressure of 120 mmHg and he was admitted for further investigation and management. Doxazosin caused his blood pressure to drop to 68/40 mmHg and was discontinued. MRI adrenals and MRA of the renal arteries did not reveal any significant abnormality. The patient did not want to remain in hospital, and was discharged with close follow-up in an ambulatory care setting. Twenty-four hour urinary noradrenaline levels were elevated at 904 nmol/24 h (70–550). Subsequently, the result of plasma metanephrines taken during his hospital admission became available and were within normal range. Following this result, he was commenced on metoprolol. Midodrine, an alpha-1 receptor agonist, was subsequently commenced for severe symptomatic orthostatic hypertension. Average 24 h blood pressure on this combination was 136/87 mmHg. Due to on-going weight loss with BMI of 15.7, he required input from dieticians.
Four months later, his weight has increased by 3 kg. His symptoms of postural hypotension are only occasional and his midodrine dose has been reduced.
Acute insulin neuritis can be precipitated by rapid improvements in blood glucose control. This resulted in acute autonomic neuropathy with marked autonomic over-activity and some features suggestive of phaeochromocytoma. Clinical history and normal plasma metanephrines helped secure the diagnosis and tailor management.

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Hypervitaminosis D, an uncommon reality!

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An 89-year-old female family physician presented to an orthopaedic surgeon with a short history of aches and pains. She was suspected to have vitamin D deficiency and was empirically prescribed three intramuscular injections of 6 million units of cholecalciferol at monthly intervals. A few days after the third dose she presented with nausea, generalised weakness, confusion and ataxia. She appeared drowsy and dehydrated. Vital parameters were normal and there were no focal neurological signs. Her calcium was 4.53 mmol/l (2.2–2.6), serum creatinine 396 μmol/l (44–100), serum vitamin D3 >160 ng/dl (30–100). She was diagnosed to have hypervitaminosis D manifesting with severe hypercalcaemia and acute renal failure.
Management included normal saline infusion, intranasal calcitonin and supportive treatment. In view of severe hypercalcemia and worsening oliguria, she underwent two cycles of haemodialysis which were complicated by disequilibrium syndrome. Serum calcium normalised and she gradually recovered and she was discharged. 3 months later calcium was 2.43 mmol/l, serum creatinine 114 μmol/l although serum vitamin D3 remained >160 ng/dl. Clinically she recovered completely and has now returned to active professional life.
With the rising prevalence of vitamin D deficiency and high cost of its biochemical confirmation there is an increasing trend amongst medical practitioners in India to prescribe vitamin D supplements empirically. This approach is generally safe in view of the wide gap between the therapeutic and toxic doses of vitamin D. However elderly patients or those with renal failure or primary hyperparathyroidism are predisposed to toxicity and may develop hypervitaminosis D especially with use of higher doses.
Our case here highlights the need to use modest doses of vitamin D therapy and to maintain clinical and biochemical vigilance for side-effects in high-risk individuals.

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P96

Simultaneous presentation of Graves' thyrotoxicosis and Addison's disease presenting as incipient adrenal crisis

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Introduction
Graves' thyrotoxicosis and Addison's disease are disorders with a strong autoimmune basis. Primary hypothyroidism and Addison's disease are recognised components of polyglandular autoimmune syndrome type II (PGA-II). Despite its autoimmune etiology, Graves disease is not commonly associated with PGA-II. We present a case of a patient with newly diagnosed Graves' disease presenting in incipient adrenal crisis due to unrecognized Addison's disease.
Case report
A 35-year-old gentleman presented with headache, paraesthesia, heat intolerance, and weight loss associated with severe fatigue, nausea and vomiting. Graves' thyrotoxicosis was suspected and confirmed biochemically (TSH <0.01 mU/l, FT_4 30.7 pmol/l, FT_3 8.6 pmol/l). He was treated with Carbimazole 40 mg once daily for a few weeks without any symptomatic improvement. He was referred to our unit with progressive weight loss, dizziness and fatigue. On re-assessment he looked unwell and was deeply tanned and hypotensive. Addison's disease co-presenting with Graves' disease was suspected. ACTH stimulation with tetracosactide (Syncathen) showed no incremental cortisol response (baseline cortisol 48 nmol/l) and elevated serum ACTH (679 ng/l) confirmed primary adrenal insufficiency. Graves' thyrotoxicosis was treated with Carbimazole. Catabolic symptoms resolved completely with glucocorticoid and mineralocorticoid replacement therapy.

Discussion
Thyroid dysfunction and Addison's disease are recognised components of polyglandular autoimmune syndrome type II (Schmidt syndrome). Primary hypothyroidism is the norm and Graves' thyrotoxicosis is very rarely recognized as part of the syndrome. It is well recognized that occult Addison's disease should be suspected in patients who fail to improve symptomatically after commencing levothyroxine for primary hypothyroidism. This case highlights the fact that patients presenting with Graves' disease and Addison's simultaneously are at risk of incipient adrenal crisis. A high index of suspicion remains the cornerstone of diagnosis.

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Severe refractory non-islet cell tumour hypoglycaemia due to metastatic colorectal carcinoma

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Introduction
Non-islet cell tumour hypoglycaemia (NICTH) is an uncommon but serious complication of disseminated malignancy. The underlying aetiology of hypoglycaemia is tumoral overproduction of IGF2, which results in stimulation of insulin receptors and increased glucose utilization. Extensive tumour burden involving the liver and adrenal glands can also cause severe hypoglycaemia.
Case report
An 80-year-old man presented acutely in an unresponsive state. Capillary glucose was recorded as 1.1 mmol/l. He had no history of diabetes and had no access to oral hypoglycaemic agents. Marked hepatomegaly, deranged liver functions (ALT 48 U/l, ALP 894 U/l, GGT 1262 U/l) and coagulopathy (prothrombin time 15.8 s) were noted. Abdominal ultrasound showed hepatic metastases and subsequent staging CT scan confirmed a primary colorectal malignancy with extensive hepatic metastases.
Severe hypoglycaemia was initially managed with a continuous 20% dextrose infusion. Diazoxide 200 mg BD was initiated but despite this and concurrent dextrose, capillary glucose remained low (<4 mmol/l). Prednisolone 60 mg once daily and subcutaneous octreotide 50 µg three times a day were subsequently initiated but despite this hypoglycaemia proved difficult to control. Serum C-peptide (<0.10 nmol/l) and Insulin (<1.0) were appropriately suppressed in keeping with NICTH. Adrenal insufficiency was excluded as a potential cause of hypoglycaemia. Despite maximal combined therapy, hypoglycaemia proved refractory and the patient succumbed to his illness.
Conclusion
NICTH is a major complication of malignancy particularly if associated with hepatic metastases. The aetiology is multi-factorial and includes reduced hepatic glycogen reserves, nutritional deficiency due to tumour induced cachexia and ectopic production of IGF2 which activates insulin receptors and promotes glucose utilization. Refractory hypoglycaemia in this context necessitates combination drug therapy but is a significant therapeutic challenge with a poor prognosis.

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Unusual presentation of central pontine myelinolysis

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Introduction
Central pontine myelinolysis, CPM classically occurs in alcoholics, malnourished and elderly, few days following rapid correction of hyponatraemia resulting in permanent neurological squeal. We described two cases of CPM occurring in alcoholics after 3 weeks of hospital admission with complete recovery of gross neurological signs.
Case 1: 24-year-old male, known alcoholic, was admitted with left hemiplegia and features of bulbar and pseudobulbar palsy with dysphagia, dysarthria and emotional lability. Admission biochemistry including serum sodium was normal apart from mildly elevated liver enzymes. No clinical evidence of infection or vasculitis with normal FBC, CRP and ESR. MRI brain showed evidence of pontine and extrapontine myelinolysis. Previous clinical records showed patient was admitted 4 weeks ago with alcohol intoxication with rapid correction of hyponatraemia with intravenous 0.9% NaCl and did not manifest any neurological deficits on discharge. He was transferred to rehabilitation unit requiring all assistance with activities of daily living including nasogatric feed. He made a complete recovery after 6 months leading an independent life.
Case 2: 55-year-old alcoholic admitted with intoxication and received intravenous 0.9% NaCl with rapid correction of sodium and developed delayed presentation of CPM with swallowing and speech difficulties and became wheel chair bound. MRI brain demonstrated typical picture of CPM. He made a complete recovery after 3 months.
Discussion
CPM is a non-inflammtory demyelinating condition affecting central pons and in 10% extrapontine sites typically involving myelin with sparing of neurons and axons. The postulated mechanism is due to rapid osmotic fluctuating from overzealous correction of sodium resulting in damage to endothelial cells with release of some myelinotoxic factors. The prognosis is poor with death or permanent neurological damage but both the cases described made complete recovery.
Recommendation
Gradual correction of SNa not more than 8 mmol/l in 24 h in high risk group.

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SIADH and bilateral adrenal infarction in a patient with the antiphospholipid syndrome

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A 75-year-old female presented with increasing drowsiness and no other specific features. Past medical history included the antiphospholipid syndrome, three episodes of myocardial infarction, left ischaemic leg, epilepsy, hypothyroidism and splenectomy. Medications included warfarin. Examination showed pulse 70/min, blood pressure 120/60 mmHg, crepitations at the left lung base, a Glasgow Coma Scale 14/15. Investigations revealed serum sodium 117 mmol/l, urea 8.8 mmol/l, serum osmolality 269 mOsmol/kg, urine osmolality 493 mOsmol/kg, urine sodium 49 mmol/l. Haemoglobin was 13.2 g/dl, initial INR 7.5. Chest X-ray showed consolidation at the left lung base. CT brain scan was unremarkable. Short synacthen test showed serum cortisol 585 nmol/l (basal) and 482 nmol/l (stimulated).
The patient was treated with intravenous antibiotics and fluid restriction with improvement in her serum sodium (128 mmol/l). The short synacthen test repeated after 1 week showed hypoadrenalism (peak cortisol 149 nmol/l). Serum ACTH was 65 ng/l (0–50), FT_4 19.8 pmol/l (11.5–22.7), TSH 0.51 mIU/l (0.35–5.50). CT showed bilateral bulky adrenals compatible with adrenal infarction with some haemorrhage. A repeat scan after three months showed significant resolution. Investigations for antiphospholipid syndrome were repeated and showed Anti-cardiolipin IgG 1902.0 GPL U/ml (0–9.9), Anti-cardiolipin IgM 119.0 MPL U/ml (0–9.9), lupus anticoagulant positive. The patient was treated with hydrocortisone and fludrocortisone and her condition improved. Her electrolytes returned to normal (serum sodium 133 mmol/l, potassium 4.7 mmol/l).
This lady therefore had evidence of SIADH which was probably secondary to respiratory infection but also had acute adrenal failure due to infarction associated with the antiphospholipid syndrome. This case illustrated the importance of considering adrenal failure due to infarction in patients with antiphospholipid syndrome who present non-specifically unwell, even when there is evidence of infection.

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P100

Incidental papillary thyroid carcinoma with primary hyperparathyroidism: two cases

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Background
Primary hyperparathyroidism (PHPT) and concomitant medullary thyroid disease is well described in literature as part of multiple endocrine neoplasia. However, coexistence of PHPT and papillary thyroid cancer (PTC) has only been scarcely documented in sporadic case reports and some surgical series.
We present two unusual cases of PHPT associated with synchronous multifocal PTC.
Cases
A 62-year-old woman with sporadic PHPT was noted to have a parathyroid adenoma by 99Tc-Sestamibi. She underwent parathyroidectomy with removal of

three parathyroid glands at neck exploration, two of which were hyperplastic. Incidentally, she was found to have metastatic follicular variant of papillary thyroid carcinoma on histology in a cervical lymph node. Further imaging showed no other metastases. She then proceeded with completion thyroidectomy with lymph node clearance followed by radioactive iodine treatment.

A 68-year-old man was referred with incidental hypercalcaemia and biochemical PHPT. 99Tc-Sestamibi scan revealed a solitary parathyroid adenoma as well as incidental left-sided cervical adenopathy. Neck ultrasound also identified a 3.6×2.1 cm enlarged left cervical lymph node and noted a contralateral 2×1.7 cm irregular thyroid nodule. Fine needle aspiration (FNA) of the thyroid lesion was insignificant. This prompted FNA of the neck node which showed metastatic papillary thyroid carcinoma. He underwent a total thyroidectomy and cervical dissection with excision of the right parathyroid adenoma. Histology confirmed PTC with capsular infiltration.

Discussion

The mechanisms underlying the association between PHPT and PTC are not established. Recent studies favour the combined use of CT–SPECT and cervical ultrasound in the first instance, coupled with FNA of suspicious lesions. These cases underline the need for a high index of suspicion for synchronous hyperparathyroidism and non-medullary thyroid cancer. Co-existence of both disease processes may complicate patient management via unrecognised thyroid cancer and the necessity for re-operative neck surgery.

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P101

Hypercalcaemia as first presentation of sarcoidosis

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A 37-year-old gentleman was admitted following a GP referral for asymptomatic hypercalcaemia. His adjusted calcium was raised at 3.27 mmol/l while phosphate level was normal. The only past medical history was borderline hypertension which was being monitored in primary care. His PTH level was suppressed at 0.43 pmol/l 1 (1.1–5.5) while 25(OH) Vitamin D was normal at 71.6 nmol/l (48–145). Twenty-four hours urinary metadrenalines were normal. His full blood counts were also normal. He was treated with i.v. fluids and i.v. Pamidronate and the hypercalcaemia responded well and his calcium levels have remained normal since then. The suppressed PTH level prompted us to look for other causes of hypercalcaemia. Familial hypocalciuric hypercalcaemia and multiple myeloma were excluded. His chest X-ray showed diffuse nodular opacities but no other specific abnormalities were present. His serum ACE level was 266 U/l (8–52) and $1,25(OH)_2$ Vitamin D was increased at >250 pmol/l (20–120). A CT scan of neck, chest and abdomen was arranged which showed subcentimeter lymphnodes at the level of carotid bifurcation and paratracheal region. There were also numerous ill defined foci throughout the lungs. It also showed an enlarged spleen (15 cm) with numerous hypo-attenuated foci in its parenchyma.

In view of splenomegaly a bone marrow biopsy was arranged which showed a reactive bone marrow with non-caseating granulomata and other changes in keeping with sarcoidosis.

His calcium levels currently remain in normal range and he is awaiting review by chest physicians.

Discussion

The incidence of bone marrow involvement in sarcoidosis is about 10%. In patients with bone marrow involvement, there is usually higher incidence of extrapulmonary manifestations, leucopaenia/lymphopaenia and anaemia.

In patients with hypercalcaemia, a normal screen for usual causes should lead to investigations for unusual causes of hypercalcaemia.

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Tumours metastatic to the pituitary gland presenting with atypical symptoms

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We report two cases with initial presentation of sudden onset ophthalmoplegia in i) a patient recently diagnosed with breast carcinoma and ii) a patient subsequently diagnosed with carcinoma lung. The first patient (68 years) was referred to the tertiary endocrine unit with a 2 weeks history of visual loss associated with 3rd cranial nerve palsy in her right eye and with a temporal hemianopia in her left eye. MRI showed an enhancing sellar and suprasellar mass. Initial biochemistry was consistent with anterior hypopituitarism. She underwent trans-sphenoidal debulking surgery. Histology confirmed a metastatic lesion consistent with a breast primary.

The second patient (49 years) presented with a one day history of left sided ptosis and retroorbital pain and symptoms of anorexia, weight loss and lethargy over a period of 1 month. He was a heavy smoker with no past medical history. Examination revealed left sided ptosis with complete ophthalmoplegia. Biochemistry was consistent with anterior hypopituitarism. MRI scan revealed an enhancing sellar and suprasellar mass. Chest X-ray showed an opacity in the left upper lobe which was confirmed by CT. He was treated with steroid and thyroxine replacement with subsequent development of polyuria and polydipsia consistent with diabetes insipidus. He underwent tras-sphenoidal debulking surgery with histology consistent with metastasis from a lung primary.

Tumours metastatic to the pituitary gland are an unusual complication of systemic malignancy. Breast and lung are the common sites of primary tumour. The most common presentation is usually diabetes insipidus which was observed in the second patient but only after steroid replacement. Sudden onset of symptoms such as headaches, ophthalmoplegia and diabetes insipidus in a patient above 45 years of age should always raise the suspicion of metastasis to the pituitary regardless of a history of malignancy.

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P103

Acute adrenal insufficiency due to bilateral adrenal haemorrhagic infarction associated with sepsis secondary to an open fracture of the ankle

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Background

Acute adrenal insufficiency is a life threatening condition. Signs and symptoms are often non-specific. The adrenal gland has the richest arterial supply of any tissue with limited venous drainage and this mismatch predisposes to haemorrhagic infarction. We present a case report of acute adrenal insufficiency due to bilateral adrenal haemorrhagic infarcts associated with an open ankle fracture.

Case history

A 52-year man fell from a ladder sustaining an open fracture of his right ankle. On presentation to A&E the wound was dressed and the fracture stabilised. X-ray revealed comminuted fractures of the distal shaft of the fibula and the distal tibia. Intravenous antibiotics and prophylactic LMW heparin were administered and baseline U&Es normal. Seventy-two hours into the admission he was taken to theatre, the wound debrided and the fibula # pinned. Anaesthetic records record some hypotension during the procedure with SBP dipping to 60 mmHg. During the subsequent days recurrent pyrexia and hypotension were noted and antibiotics continued. His Na gradually fell with a nadir at day 9 of 122 mmol/l. This responded to intravenous therapy and at a 2nd operation on day 13 the tibial # was pinned. Serum Na was again noted to fall over the next few days with a nadir of 124 mmol/l on day 23. The patient complained of intermittent abdominal pain and began hallucinating on day 20. Enterobacter cloacae was cultured from the wound. Serum cortisol was measured on days 23 and 24 being undetectable on both occasions. His platelets had dropped to 56. A diagnosis of acute adrenal insufficiency was made and high dose intravenous hydrocortisone administered. Clinical improvement was evident within 48 h. Baseline pituitary tests were unremarkable. CT scans showed enlarged heterogeneous adrenal glands consistent with bilateral haemorrhagic infarction.

Conclusion

Acute adrenal insufficiency due to bilateral adrenal haemorrhagic infarction is a rare event that can have catastrophic consequences if not diagnosed and treated in a timely manner. Our patient presented with a common orthopaedic injury and an inpatient course characterised by pyrexia, low BP and hyponatraemia, in themselves unremarkable and frequently associated with trauma/sepsis. A high index of suspicion in a context of slow deterioration lead to further investigation and the additional diagnosis being made.

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P104

Ectopic thyroid tissue presenting as metastatic follicular cancer

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We present the case of a 73-year-old woman, who presented with ascites and a history of left radical hemithyroidectomy for localized follicular thyroid

carcinoma (FTC) 28 years ago. Computed tomography (CT) scanning of her body revealed extensive metastatic lesions. An omental biopsy showed features suggestive of thyroid follicular epithelial cancer. Serum thyroglobulin was raised at 127 μg/l. She died before the biopsy result was received. Two years before presentation, a bilobed nodule was noted in the superior mediastinum measuring 3.5×1.5 cm, on a CT chest organized by her primary care physician to investigate breathlessness. A thyroid uptake scan that followed had shown normal uptake in the right thyroid remnant and a high uptake in this structure, suggestive of ectopic thyroid tissue. No further action was taken at the time. A retrospective review of her thyroid function tests showed that serum thyrotropin (TSH) had never been suppressed and she had untreated hypothyroidism 3 years before her presentation. We suspect that she may have harboured FTC cells from the primary lesion in the ectopic gland or developed a *de-novo* lesion within it that spread due to inadequate TSH suppression. A timely diagnosis by a positron emission tomography (PET) or radio-iodine scan would have provided grounds for consideration of curative or palliative radio-iodine ablation.

Thyroid cancer, the commonest endocrine cancer, is relatively rare in comparison to other cancers (<1% of all cancers). It is therefore least suspected in an individual presenting with metastatic disease and an unknown primary. Follicular thyroid cancer is the second most common differentiated thyroid cancer. Current practice involves total thyroidectomy (older patients, high-risk cases), followed by radio-iodine ablation and long-term TSH suppression. This case emphasizes the importance of long-term follow up of this condition, in order to monitor for recurrence and to maintain TSH suppression.

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A peculiar case of a dog bite

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A 59-year-old gentleman presented to the Medical Admission Unit with facial and ankle oedema following a dog bite. He did not have any significant past medical history. Initially, he was treated for angioedema. His oedema worsened to anasarca, blood pressure rose and was found to be hypokalaemic. Echocardiogram showed a normal left ventricular ejection fraction. Urine protein creatinine ratio was <0.5 g/24 h. Vasculitic and autoimmune screen were negative. Eight weeks later, he was noted to have a Cushingoid appearance, ongoing persistent hypertension with hypokalaemia, as well as a new diagnosis of diabetes mellitus. He had pulmonary oedema and ascites on clinical examination. He was treated with furosemide, spironolactone and insulin.

Subsequent biochemical investigations showed high random cortisol (2202 nmol/l) unsuppressed with low dose dexamethosone test, low ACTH (<5 ng/l) and high ACTH precursor (POMC) (610 pmol/l). He also had elevated fasting gut hormone levels – CART (469 pmol/l), chromogranin A (247 pmol/l), chromogranin B (216 pmol/l).

Imaging with MR and CT scanning showed liver metastases and bilateral adrenal nodules (largest measuring 27 mm). Further investigations showed these were somatostatin receptor negative. Liver biopsy demonstrated grade 3 poorly differentiated neuroendocrine cancer. PET/CT scan revealed high metabolic activity within the tail of pancreas, bilateral adrenal lesions and suspicious malignant liver lesions.

The patient underwent bilateral adrenalectomy, distal pancreatectomy and splenectomy. Pancreatic histology confirmed grade 3 poorly differentiated neuroendocrine cancer. Post operatively, his blood pressure, serum potassium and blood glucose have normalised.

At the local neuroendocrine multidisciplinary team meeting, chemotherapy has been recommended, and is awaiting assessment for the same.

Conclusion

This case denotes rapidly deteriorating ectopic Cushing's syndrome following a misleading history of a dog bite which delayed diagnosis. It demonstrates the importance of correlating endocrine and biochemical findings with a clinical diagnosis.

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P106

Management of diabetic ketoacidosis in a district general hospital: a 3 years retrospective audit

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Objectives

Diabetic ketoacidosis (DKA) remains a life-threatening emergency. An updated protocol was introduced in Sligo general hospital in 2009 to standardise DKA management. The aim of this study was to audit current practice against the protocol standards.

Methods

Clinical notes of all adult patients admitted with DKA between July 2009 and June 2012 were analysed and retrospectively audited against the protocol.

Results

Fifty-six episodes of DKA were identified in 36 patients. 57.1% ($n=32$) were males, and the median age was 28.5 years. 87.5% of episodes occurred in patients with type 1 diabetes ($n=49$). Infection was the main precipitating factor in 33 episodes (58.9%) followed by non compliance 19.6%, new onset diabetes 14.3%, and alcohol excess 7.1%. The majority of patients were managed in a general medical ward (58.4%, $n=33$), 25% ($n=14$) were managed in ICU, and nine patients (16.1%) in HDU. There were no deaths and the median length of stay was 4 days. Fluids were commenced within 60 min of arrival to ED in 91% ($n=51$) of episodes and the mean time to commence fluids was 30.3 ± 3.6 min. Insulin was started within 60 min in 46 episodes (82.1%), with a mean time of 48.5 ± 5.65 min. Potassium was inadequately replaced in 30.4% ($n=17$), and mild hypokalaemia (K^+3.0–3.5) occurred in 10 episodes (17.9%). Long acting insulin was inappropriately stopped in 74.3% of admissions. The frequency of biochemistry and blood gas measurements followed the protocol in only 37.5 and 51.8% respectively. 93% ($n=52$) of admissions care were taken over by the diabetes team, and 89.3% ($n=50$) received education by the diabetes nurse pre discharge.

Conclusion

Although insulin and fluid replacements were generally in line with the recommendations, potassium replacement, continuing basal insulin, biochemistry and blood gas monitoring were inadequate. Emphasizing the importance of these parameters, especially to the junior doctors, is essential to improve care standards.

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P107

Unusual presentations of adrenocortical tumours

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Background

Adrenocortical tumours (adenoma or carcinoma) present in well-recognised ways: hormones excess (Cushing's, Conn's, virilisation) or hormonally silent with symptoms of mass effect, or found incidentally on imaging. We present 3 cases of adrenal tumours, referred to our regional adrenal multidisciplinary meeting with unusual presenting features.

Case 1: post-menopausal bleeding

A previously well 57-year-old female presented with vaginal bleeding 5 years after completing menopause. She was not using exogenous oestrogen. Hysteroscopy demonstrated endometrial hyperplasia. Subsequently, she developed right-sided abdominal pain. Imaging revealed 6 cm invasive right adrenal mass. Serum oestradiol and 17β-oestradiol were elevated. Oncological right adrenalectomy was performed. Histology confirmed invasive adrenocortical carcinoma. Serum oestradiol fell to post-menopausal levels. No further vaginal bleeding was noticed.

Case 2: polycythaemia

A previously well 49-year-old female was noted to have hypertension and polycythemia (haemoglobin 17 g/dl). Venesection (following a haematology opinion) was unsuccessful in controlling polycythaemia. Subsequently, significant weight gain and increased body hair over the preceding year were noted. She had features of Cushing's syndrome, marked hirsutism and increased muscle tone. Investigations showed high serum testosterone (23 nmol/l) and elevated non-suppressible serum cortisol. Imaging showed 3.5 cm left-sided well-circumscribed adrenal lesion. It was treated with laparoscopic left adrenalectomy. Histology demonstrated oncocytic adrenocortical adenoma. Post-operatively, cortisol and testosterone levels normalised and polycythaemia resolved.

Case 3: hypertension and low serum aldosterone

A 27-year-old female presented with 6-month history of hypertension, hypokalaemia (2.8 mmol/l) and low serum aldosterone (86 pmol/l, range 100–450 pmol/l). She subsequently developed abdominal pain. Imaging revealed 10 cm left adrenal lesion. Serum cortisol was suppressed (<30 nmol/l) after overnight dexamethasone (1 mg). Urinary and plasma catecholamines/metanephrines were normal. Urinary steroid profile demonstrated marked increases of metabolites of 11-deoxycortisol(S) and 11-deoxycorticosterone. Histology from

the oncological resection of the mass revealed adrenocortical adenoma. Hypertension resolved post-operatively.
Discussion
These three cases represent rare presenting features of adrenal tumours.
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P108

An audit of the diagnosis and management of hypogonadism in adult men

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Introduction
Symptomatic hypogonadism affects 5–6% of men aged 30–79 years and is associated with increased morbidity and mortality. There are currently no trust guidelines for the diagnostic workup of these patients.
Methods
Retrospective analysis was performed of diagnosis and assessment of adult men with low serum total testosterone (TT) and compared with clinical practice guidelines of The Endocrine Society. We identified all patients with TT below normal range ($<$9.9 nmol/l) carried out at the trust between July and November 2010 and excluded patients with known hypogonadism or prostate cancer. First 50 cases were evaluated in detail. Investigation results were retrieved using electronic records and clinic letters.
Results
In total, 247 cases with low TT were identified. Among 50 patients (mean age 63, range 34–94 years) 84% (42/50) had a valid indication for measurement. Mean time ($\pm$S.D.) of measurement was 11:18am $\pm$ 142 min. Fifty-eight per cent (29/50) had a confirmatory repeat measurement, 42% (21/50) had sex hormone-binding globulin measured and 52% (26/50) had gonadotropins measured, of which 69.2% (18/26) were low/normal and 26.9% (7/26) were high. Of those with low/normal gonadotropins, 50% (9/18) had complete pituitary function testing (cortisol, prolactin, free thyroxine). Testosterone replacement therapy was commenced in 44% (22/50). Pre-treatment, 45.5% (10/22) had PSA, 27.3% (6/22) had liver function, and 27.3% (6/22) had haematocrit measured. Within six months after starting treatment, 86.4% (19/22) were reviewed in clinic, 63.6% (14/22) had repeat TT and 50% (11/22) had repeat haematocrit.
Conclusion
There are pitfalls in our current practice in both diagnosis and assessment of hypogonadism. Morning TT is measured later than recommended and often not repeated, resulting in potential over-diagnosis. Lack of gonadotropin measurement has serious implications for missing potential aetiology. Pre-treatment assessment and treatment monitoring must be improved to ensure patient safety. We are currently writing trust guidelines to address these issues.
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P109

Pituitary tuberculosis

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A 28-year-old man of Asian origin presented with fever, cough and weight loss over 4 weeks. He had symptoms of worsening headaches and vomiting. The chest X-ray was normal and due to persistent headache and vomiting a lumbar puncture was performed following a normal CT head scan. The CSF findings confirmed the diagnosis of tubercular meningitis and the patient received a complete course of anti tubercular treatment. Six months later he was readmitted with visual blurring, a diagnosis of optic neuritis was made and he was treated with steroids. The patient re-presented in a few months with symptoms of polyuria, polydipsia and excessive tiredness. The biochemistry demonstrated evidence of secondary hypothyroidism and subsequent testing confirmed anterior hypopituitarism. The patient was commenced and hydrocortisone, thyroxine and testosterone. Symptoms of diabetes insipidus resolved following hormone replacement and a water deprivation test undertaken at a later date was completely normal.
Pituitary tuberculosis is extremely rare and a reported incidence of 4% in patients with tuberculosis has been quoted in the literature. It accounts for $\sim$0.15% of all intracranial tumours and 0.6% of all intracranial TB. Endocrine dysfunction has been reported to occur in patients with tubercular meningitis and the most commonly reported endocrinopathies include adrenal insufficiency, central hypothyroidism and hyperprolactinemia. Hypopituitarism has been reported to occur years after recovery from the illness in some patients who had suffered from tubercular meningitis in their childhood. Trans-sphenoidal biopsy has been considered to be essential to establish the diagnosis. Anti tubercular treatment regimes used in CNS tuberculosis have been used to effectively treat pituitary tuberculosis. It is important for the endocrine fraternity to recognise pituitary tuberculosis and tubercular meningitis as rare causes of hypothalamic pituitary insufficiency as anti tubercular treatments are curative.
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The difficulties in diagnosing and treating phaeochromocytoma in a patient with multiple co-morbidities

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A 33-year-old lady reported a 6 months history of sweating and worsening palpitations especially after taking sotalol. She had a history of congenital heart disease (double inlet left ventricle, pulmonary valvular stenosis, ventricular septal defect, and Fontan connection surgery at 18 years); Blue Bleb Syndrome with chronic gastrointestinal blood loss, and recurrent pulmonary emboli. She required long-term warfarin treatment and regular blood transfusions. She was found to have new hypertension. Her cardiac performance and right ventricular ejection fraction (44%) had reduced which serves her systemic circulation. Her symptoms and modestly elevated urinary noradrenaline levels were initially felt to be in keeping with worsening intrinsic cardiac function (596–892 nmol/24 h; $n=50$–560). A clonidine suppression test and genetic screening were negative. However plasma normetanephrines were significantly elevated at 5415 and 5661 pg/ml ($n<1180$) and a MIBG with spect CT scan (meta-iodobenzylguanidine with single-photon emission computed tomography) demonstrated a hot spot at the left para-aortic region. She had a prolonged admission for withdrawal of sotalol and frusemide and introduction of alpha-blockade which she tolerated well. Inputs were sought from cardiac, endocrine and vascular surgery, anaesthetics, cardiology, haematology and gastroenterology. She proceeded to elective surgery which was uncomplicated apart from modest postoperative bleeding. Follow-up urinary catecholamines normalised and the patient's presenting symptoms partially abated. This case illustrates the difficulty of diagnosing primary catecholamine excess in the setting of severe cardiac disease and also the success of the multidisciplinary approach for patients with multiple threatening co potentially life-morbidities.
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P111

Adrenal incidentalomas: who requires further testing?

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Adrenal incidentalomas are common and guidelines recommend testing to exclude functioning lesions and malignancy. Their increasing prevalence results in a number of investigations usually conducted in the Endocrinology clinic.
In 2011 we audited the prevalence and management of adrenal incidentalomas identified on abdominal CT imaging over one calendar month in our centre. Consequently, a decision pathway for new adrenal lesions was introduced in the Radiology department. This pathway is based on lesion imaging characteristics and a brief clinical assessment. A year later we re-audited the local practice.
690 CT scans were reviewed in 2011 and 1264 in 2012. In 2011, 32/690 (4.64%) scans with adrenal lesions were identified and in 2012, 85/1264 (6.7%). In both the 2011 and the 2012 cohorts, the majority of patients with adrenal lesions found on imaging, had a known malignancy or adrenal metastasis and were under Oncological care (13/32, 40.6% in 2011; 42/85, 49.4% in 2012). Excluding patients with malignancy and other radiological diagnosis, 17 (2.46%) patients in 2011 and 26 (2.01%) in 2012 with adrenal incidentalomas were identified. Of those, 1.01% in 2011 and 0.95% in 2012 had newly identified incidentalomas. Only a minority of patients with incidentalomas had testing to exclude a functional lesion (5/17, 29.4% in 2011; 4/26, 15.4% in 2012). Hounsfield units were reported in 9/17, 52.9% in 2011 and in 8/26, 30.8% patients with incidentalomas in 2012. There was inconsistent terminology in reporting adrenal lesions.
We support comprehensive reporting of incidentalomas and a selective testing strategy.
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P112

GH therapy in adults with Prader–Willi syndrome

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Prader–Willi syndrome (PWS) is a genetic syndrome caused by a disorder of chromosome 15 (q11–13) are deleted or unexpressed on the paternal chromosome. It affects both sexes equally.

Characteristics of PWS include hypotonia, hyperphagia with excessive weight gain, short stature delayed puberty and hypogonadism. Individuals with PWS are at risk of learning and attention difficulties.

Most adults with PWS have deficiencies of GH or the GH/IGF axis and suffer similar body composition abnormalities compared with both children with PWS and adults with GH deficiency.

GH replacement in children with PWS is well accepted and extensively used. Studies suggest that GH therapy may be beneficial in adults with PWS GH use in PWS adults remains controversial due to effects of GH on glucose homeostasis, the metabolic syndrome (MS), and other obesity- and age-related comorbidities in overweight and obese PWS adults. We describe a series of adult patients treated with GH at St George's Hospital. Theses patients showed initial weight loss with stabilisation of weight. There was improvement in their behaviour with increase in energy levels and better quality of life. The GH was well tolerated with increase in IGF1, and without deterioration in glucose metabolism. One patient had significant GH-mediated water retention during dosage optimization. This suggests judicious GH initiation, dosage adjustment, and careful monitoring.

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P113

Primary hyperparathyroidism in pregnancy: a conservative approach

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Primary hyperparathyroidism (PHP) during pregnancy is associated with high risk of maternal, foetal and neonatal mortality. Maternal and foetal complications have been reported in 67 and 80% of cases respectively. Guidelines for the management of PHP in adults exist, but there is no clear consensus regarding optimal management of PHP during pregnancy. We describe a case of PHP managed conservatively during pregnancy, resulting in the delivery of a healthy baby.

A 32-year-old lady with a known history of gallstones was admitted with cholecystitis. She was noted to have an elevated serum corrected calcium of 2.64 mmol/l (2.1–2.6 mmol/l). Further investigation revealed an elevated plasma parathyroid hormone concentration of 140 pg/ml (10–85 pg/ml), vitamin D level of 73 nmol/l (>50 nmol/l) and 24 h urinary calcium level of 4.51 mmol/l (3–9 mmol/l), consistent with a diagnosis of PHP. She had no past history of fractures, renal calculi or chronic kidney disease. DEXA scan confirmed osteopenia. TcMIBI scan localised a parathyroid adenoma and she was referred for consideration of surgery.

When she attended the surgical clinic, she was 9 weeks pregnant. After discussion with the patient, a conservative approach was favoured, delaying surgical resection until after delivery. Surgery in the second trimester was considered as a possible option should she become symptomatic.

She adhered to a eucalcaemic diet and maintained adequate hydration. She was closely monitored with monthly bone profile measurement. She remained asymptomatic throughout pregnancy, foetal growth was satisfactory and serum calcium ranged from 2.52–2.87 mmol/l. A healthy baby, weighing 3.1 kg was delivered at 38 + 1 weeks gestation. Maternal and neonatal calcium levels were monitored and were satisfactory following delivery. There were no signs of neonatal hypocalcaemic tetany. A left parathyroid adenoma was excised at 6 months *post-partum*.

This case demonstrates the importance of an individualised management plan, based on severity of disease, symptoms and gestational age. Increased awareness and careful management are key to decreasing complications associated with the condition.

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P114

Audit of the management of primary hyperparathyroidism at Watford general hospital

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Introduction

Primary hyperparathyroidism (PHPT) can only be cured by parathyroidectomy (PTX) but mild and asymptomatic cases can often be managed conservatively. We carried out a retrospective audit of patients attending the Endocrine clinic at Watford to determine how our practice compared with the different guidelines for surgical referral published in the USA and the UK.

Method

A search was carried out of clinic letters from Jan 2010 to Oct 2011. Additional data were gathered from the hospital biochemistry results system. We determined the number of patients who fulfilled either of two sets of criteria for surgery and whether this had taken place. UK indications for PTX were: serum calcium >3.0 mmol/l, renal calculi, acute pancreatitis, age <50 years, osteoporosis at hip or spine, and 24-h urine calcium excretion >10 mmol/day. USA surgical criteria for asymptomatic PHPT were serum calcium >2.8 mmol/l, GFR <60 ml/min, osteoporosis and age as above.

Results

Ninety-seven patients were identified with PHPT. Thirty-two of these patients were referred for PTX. Fifty-five patients had indications for surgery according to UK guidelines and 29 of these (53%) had so far been referred. Of those not referred the indication for surgery in 16 was osteoporosis only. Seventy-one patients fulfilled USA criteria for PTX and 31 of them (44%) had been referred. Twelve patients not yet submitted to PTX had a GFR <60 ml/min. Mean age of all patients not referred was 72.6 years.

Conclusion

Our practice in this cohort was relatively conservative. Approximately half of patients who fulfilled some criterion for surgery (USA or UK) had not been referred. Reasons for this included age, stable disease, co-morbidities and patient choice. Further explanation from retrospective analysis will be available and a prospective audit is also underway.

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P115

Potential role of antituberculosis treatment as cause of severe hypocalcaemia immediately following total thyroidectomy

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We report potential effects of antituberculosis (TB) treatment on vitamin D metabolism in the immediate postoperative period following total thyroidectomy. A 50-year woman of Chinese origin was incidentally found to have papillary thyroid carcinoma with local nodal metastasis following FDG–PET imaging done to assess nature of lung nodules on chest X-ray and CT chest. The nature of lung lesion was uncertain and commenced on empirical TB treatment (four drug regimen). At this stage 25-hydroxy vitamin D (25-OHD) level was 71 nmol/l and calcium 2.20 (ref. range 2.1–2.55 mmol/l). One month later she had total thyroidectomy and level six neck dissection. She developed severe symptomatic hypocalcaemia in the immediate postoperative period with calcium 1.70. Symptoms improved with emergency treatment but remained symptomatic for 4 days requiring continuous calcium infusion. During this time she was commenced on alphacalcidol and oral calcium, and her calcium level stabilised by day 5. PTH level was 0.1 (ref. range 1.6–6.9 pmol/l) at discharge and improved to 0.4 within 2 weeks, while her calcium remained stable and was able to reduce dose of alfacalcidol.

TB treatment, especially rifampicin is reported to cause significant reduction in 25-OHD within few weeks after starting treatment. Several reports in the literature suggest adverse effects of TB treatment on vitamin D metabolism. It is reported that rifampicin binds to steroid and xenobiotic receptor (SXR) which mediates 24-hydroxylase activity leading to increased clearance of vitamin D (1). We suggest that treating clinicians are aware of the need for closer monitoring of patients on TB treatment undergoing thyroid surgery and perhaps consider monitoring and correction of vitamin D insufficiency preoperatively to avoid complications of severe hypocalcaemia in the immediate postoperative period.

Reference
1. Zhou C *et al.* Steroid and xenobiotic receptor and vitamin D receptor crosstalk mediates CYP24 expression and drug-induced osteomalacia. *JCI* 2006 **116** (6) 1703–1712.

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P116

Pelvic pain in a type 2 diabetes patient

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Objective
To present a patient with long standing type 2 Diabetes complaining of chronic pelvic pain, due to an uncommon cause of bilateral vas calcification.
Methods
Clinical, laboratory and radiographic data are reported on a 62-year-old diabetic presenting with chronic pelvic pain.
Results
A 62-year-old man with a history of 17 years of diabetes presented with chronic dull aching, non radiating pain in the pelvis and in the region of sacal sulcus below 5th lumbar vertebra. There was no history of fever with chills and sweats, dysuria, urgency, frequency of urination. Pain was not aggravated during intercourse. There was no past history of sexually transmitted disease, frequent and extramarital sexual encounters, chronic kidney disease. Complete blood picture and routine urine examination did not reveal any evidence of infection or proteinuria. Fasting and post prandial blood sugars were 104 and 136 mg/dl with HbA1c at 6.7%. Other blood parameters including lipid profile, renal and liver function tests, serum calcium, phosphorous were all within normal limits. X ray showing anteroposterior view of pelvis revealed bilateral serpentine structures with symmetric and regular vas deferens calcification involving vas calcification.
Discussion
The causes of bilateral vas calcification include degenerative changes due to ageing, diabetes mellitus, end stage renal disease with secondary hyperparathyroidism. They give rise to regular calcifications within the muscular components of the vas with preservation of luminal patency. Causes of unilateral vas calcification include inflammatory conditions like tuberculosis, gonorrhea, syphilis, schistosomiasis, and chronic non-specific urinary tract infections. The calcifications are intraluminal and irregular leading to partial or complete occlusion of the lumen. Vasa differentia may calcify after relatively short duration of diabetes if the disease starts after the age of 40, whereas if the disease occurs before the age of 40, it has usually been present for at least 15 years before calcification is noted. Diabetes accelerates the process of senescent calcification of the vas deferens by augmented expression of several bone-associated proteins (e.g. osteopontin, bone sialoprotein, alkaline phosphatase, type one collagen, osteocalcin) that facilitate or regulate the calcification process. In addition uremic serum upregulates osteoblast transcription factor Cbfa 1 and osteopontin expression. Diabetic patients with vasal wall calcification may also develop failure of emission, where no sperm reach the posterior urethra due to aperistalsis of the vas deferens.
Conclusion
Type 2 diabetic subjects with long standing pelvic pain and without any elicitable cause should be evaluated for this uncommon etiology of vas calcification.

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P117

Clinical practice hyperaldosteronism: a misleading adrenal tumor

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Background
A 58-year-old male known to have hypertension since 2002 was initially treated with Furosemide and Bendroflumethiazide but was stopped due to hypokalemia. Blood pressure was controlled with Ramipril, Atenolol, Doxazocin and Amlodepine. He was found to be persistently hypokalemia ranging from 2.7 to 3.3 mmol/l.
Investigations
Aldosterone: 250 ng/l, renin: <2.3 mU/l, aldosterone:renin ratio: >108, 24 h urine catecholamines $\times 2$: normal CT scan (Oct 2010) – 8 mm right adrenal adenoma MRI (Jan 2011) – right adrenal gland was not clearly seen due to movement artefact.

Table 1 Adrenal vein sampling

	Aldosterone	Cortisol	Androstenedione
Right adrenal vein	411	559	0.74
Left adrenal vein	$>34\,340$	8809	>70

Discussion
This man had biochemical evidence of primary hyperaldosteronism. Initial CT scan showed evidence of right adrenal adenoma but adrenal vein sampling showed that the hyperaldosteronism originated from the left adrenal gland. This case demonstrates the value of using adrenal vein sampling to localise the hyperfunctioning gland in the absence of definitive and potentially misleading adrenal imaging.

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P118

Bilateral adrenal calcification caused by a previous *Streptococus mitis* septicaemia

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This 23 years old man was noted incidentally to have bilateral adrenal calcification on CT scan of his abdomen for chronic abdominal pain. He had normal growth and milestones with no neonatal events. Aged 10 years he was admitted briefly to a high dependency unit with circulatory shock and hyperthermia, with isolation of Streptococcus mitis from blood culture. One year later he was noted to have bilateral adrenal calcification on abdominal XR, and a short Synacthen (250 μg) test was considered normal (baseline cortisol of 303 nmol/l rising to a peak of 543 nmol/l). Current investigations reveal normal adrenal function including a Short synacthen test; which revealed normal response with base line cortisol of 440 nmol/l rising to a peak of 620 nmol/l. Renin was 20 mU/l and ACTH was 25.0 ng/l.
This case illustrates bilateral adrenal calcification without enlargement of the glands, possibly after an episode of adrenal hemorrhage at the age of 10 years, presumably due to an episode of septicaemia caused by Streptococcus mitis.Normal adrenal function has been maintained for 13 years. Waterhouse–Friderichsen syndrome is classically due to meningococcal septicaemia,but may be precipitated by *S. pneumoniae* and β haemolytic streptococcus group A infection Hemorrhage is the most common cause of adrenal calcification. In 20% of the cases it can be bilateral, Most (90%) of the adrenal tissue must be destroyed for adrenal insufficiency.

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P119

A question of GH deficiency or not

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We present a 45-year-old lady who initially presented with neurological symptoms and MRI was noted to show asymmetry of the pituitary gland. There were no endocrine symptoms. In 2011 she developed some visual symptoms (not typical of pituitary disease) and was referred to the ophthalmic department. MRI scan showed an 11 mm right-sided pituitary lesion and she was referred to the endocrine department. She gave a 2-year history of feeling increasingly unwell with back pain, sensory disturbance in her limbs, nausea and left-sided frontal headache. There were no symptoms or signs of pituitary dysfunction. Initial tests showed an elevated IGF1 38.9 nmol/l (13.0–35.0) but subsequent GH suppression test was normal (nadir GH 0.12 μg/l). Repeat IGF1 was normal (29.3 nmol/l). An insulin stress test (IST) with nadir glucose of 1.1 mmol/l revealed peak cortisol of 477 (NR >550) and peak GH of 1.28 μg/l (>6.6, 3.3-6.6 equivocal) demonstrating partial hypopituitarism. The patient began hydrocortisone replacement therapy. The patient underwent hypophysectomy May 2011. Histology showed a plurihormonal secreting tumour. Operation was difficult due to the tough nature of the adenoma and later MRI revealed residual adenoma. She subsequently had pituitary irradiation early 2012. The patient continued to feel unwell complaining of lack of concentration and tiredness. Adult GH deficiency assessment (AGHDA) was 23/25. Repeat IST in September 2012

demonstrated cortisol and GH deficiency (peak cortisol 396 nmol/l and peak GH 0.97 μg/l). She was therefore started on hydrocortisone and 0.1 mg GH treatment. However IGF1 level taken at time of IST was noted to be above the normal range (35.1 nmol/l). In conclusion we present a patient with conflicting results on GH status with repeatedly elevated IGF1 levels with GH deficiency on IST.

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P120

Ectopic ACTH syndrome as a presenting symptom of bronchogenic carcinoma

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Introduction
Ectopic ACTH syndrome (EAS) is associated with small cell carcinoma of the lung. It is reported as a rare condition. Here we report three cases of undiagnosed bronchogenic carcinoma who presented with EAS within a period of 12 months.
Case 1
67-year-old lady, smoker, presented with severe proximal myopathy of 4 week duration. Clinically she appeared cushingoid. Newly diagnosed Type two diabetes. Lab tests – potassium 2.3 mmol/l, bicarbonate 37 mmol/l. CXR showed abnormal shadow at left hilum. 0900 h cortisol 1406, ACTH 80 (normal 0–40). CT scan of thorax/abdomen/pelvis revealed large left hilar mass, infiltrating left main bronchus, multiple hepatic and bilateral adrenal metastatic lesion.
Case 2
A 53-year-old female smoker presented with shortness of breath and weight loss. Her blood pressure had become uncontrolled despite being on several antihypertensives. Newly diagnosed diabetes.She was cushingoid and had severe proximal myopathy. Lab test – potassium was 2.5 mmol/l, bicarbonate 35 mmol/l. Glucose – 30 mmol/l. Chest radiograph – right lower lobe consolidation. An early morning cortisol >1600l/l (normal range 450–700 nmol/l). ACTH 96 ng/l (normal range <80 ng/l) in presence of high cortisol. Bronchoscopy-bronchogenic carcinoma. Histology – small cell lung carcinoma (SCLC).
Case 3
A 70-year-old male, non-smoker presented with shortness of breath for 6 months. He had dysphagia, weight loss and hoarseness of voice. Lab tests potassium of 2.5 mmol/l, with normal sodium, urea, creatinine. Bicarbonate 35 mmol/l. Chest radiograph – left hilar mass, left basal consolidation. 0900 h cortisol sample 1595 nmol/l (normal 450–700 nmol/l).
CT staging-multiple masses within the mediastinum and left hilum. There were multiple liver metastasis. Bronchoscopy-tumours in the left upper lobe. Histology-small cell lung carcinoma.
Discussion
EAS is reported as a rare diagnosis in SCLC (5% of all cases). Here we report three cases who presented with EAS in previously undiagnosed bronchogenic carcinoma. The clue to diagnosis was unexplained hypokalaemia and alkalosis in all cases which was confirmed by biochemical testing. Early recognition of the condition allows symptom control to improve quality of life but does not affect prognosis.

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P121

Secondary diabetes due to phaeochromocytoma

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Case: A 73-year-old Pakistani gentleman with known hypertension and type two diabetes mellitus initially presented to hospital hypotensive, with epigastric pain and vomiting. An abdominal CT confirmed no abdominal aortic aneurysm, but discovered incidental findings of a 5.5×3×3.7 cm left-sided adrenal mass and a 1.5 cm gallbladder stone. He was treated for suspected cholecystitis and 24-h urinary free catecholamines requested. Unfortunately he was lost to follow-up.
He was admitted few years later with symptoms of vomiting and bilateral flank pain. On this occasion he was hypertensive (211/123). Urine dipstick was positive for leucocytes, protein and blood with his biochemistry mimicking an acute kidney injury pattern. Given the raised WCC (24.1) and renal angle tenderness, he was treated for pyelonephritis. A repeat CT abdomen confirmed the previous adrenal mass with no change in size. Repeat urinary free catecholamines were elevated. The urinary free adrenaline level was 1470 nmol/coll (8–101) and noradrenaline level 748 nmol/coll (82–650). Both noradrenaline and adrenaline creatinine ratios were also raised. An MIBG scan demonstrated increased uptake in the left adrenal mass. A diagnosis of phaeochromocytoma was confirmed.
Following adequate adrenergic block with phenoxybenzamine, he underwent an elective laparoscopic left-sided adrenalectomy. He recovered well post-operatively with his urinary catecholamines both within normal range. Histology confirmed the mass was a phaeochromocytoma. Following surgery both his blood pressure and capillary blood sugars normalised post-operatively and therefore no longer required insulin or anti-hypertensives.
Discussion
Phaeochromocytomas are commonly associated with impaired glucose tolerance through excessive production of catecholamines, hormonal antagonists of insulin. If a phaeochromocytoma is diagnosed and managed appropriately, it can lead to a significant reduction in the patient's insulin requirement and even resolution of their diabetes. Clinicians should maintain a high level of suspicion of secondary reversible causes when managing diabetic patients.

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P122

Absent thyroid with GH deficiency

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Background
Thyroid dysgenesis (TD) represents a heterogeneous group of conditions and accounts for 85% of cases of congenital hypothyroidism (CH). This can be due to abnormal gland organogenesis which results in thyroid agenesis (35–40% of the cases), thyroid hypoplasia (5%) and thyroid ectopy (30–45%).
Causes of CH can be classified into: one. dyshormonogenesis usually associated with goiter, caused by mutations in the genes coding for the proteins responsible for thyroid hormone synthesis; two. dysembryogenesis or dysgenesis, that may be due to: i) inactivating TSHR mutations; ii) genetic mutation affecting the thyroid transcription factors.
We report a case of 54 years old lady who presented to our endocrine clinic with hypothyroidism on thyroxine replacement, sensorineural hearing loss, short stature (9°centile) and obesity (BMI 40.3). She did not have any learning difficulties. She was diagnosed hypothyroid at 14 years of age. She had no significant past medical or family history. Her thyroxine was stopped for 3 weeks and investigations were carried out. She tolerated discontiuation of thyroxine well. Her TSH was 84.6 mU/l (0.4–5.0), FT_4 was 4.9 pmol/l (10–23) and FT_3 was 1.6 pmol/l (3.5–6.5) suggesting primary hypothyroidism. She was also found to have GH deficiency on provocative test. Thyroid uptake scan revealed absent thyroid gland. MRI of the pituitary showed normal gland. In view of deafness CT scan of the inner ear was done and it was normal. Genetic test for Pendred syndrome was negative. She has been started on thyroxine and GH replacement.
Interpretation
We report a case of primary hypothyroidism due to hypoplastic/absent thyroid tissue associated with GH deficiency, sensorineural deafness and normal mental development. Familial occurrence of GH deficiency and primary hypothyroidism has been reported in three male siblings in the literature but thyroid absence with GHD has not been reported to our knowledge.

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P123

Degree of bone mineral density improvement in patients with osteoporosis on current NICE recommended treatments: an audit

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Aims
To identify any improvements in BMD on NICE recommended treatments in patients with osteoporosis. Also to identify how many patients were taking calcium and vitamin D.
Methods
Retrospective audit of 31 patients who had a DEXA scan between June 2011 and October 2012 at Birmingham City Hospital. Patient information was gathered from electronic data base (Clinical Data Archive). Inclusion criteria was at least two DEXA scan *T*-scores results, one before and after at least 1 year of treatment.
Results
63% were females, mean age 54 years, 42% were White British and mean

duration of osteoporosis 4 years. Cause of osteoporosis was varied. 24/31 patients met audit standards. 70% of patients were taking Alendronic acid. 100% of the patients with osteoporosis were taking calcium and vitamin D supplements. 58% had an improvement in spine and femur *T*-score (mean improvement 1.2 s.d.). three patients had a deterioration DEXA *T*-score and treatment was changed according to NICE guidelines to Raloxifene (2) and to Strontium Ranelate (1). three patients had a deterioration in femur *T*-score only and four had a deterioration in the spine *T*-score.

Conclusions

Nearly 2/3rd of patients (58%) with osteoporosis on NICE recommended treatments experienced significant improvements in spine and femur BMD. 100% of the patients were taking calcium and vitamin D according to recommended guidelines. This audit shows that current treatment for osteoporosis is effective and most patients adhere to their drugs although compliance was difficult to ascertain from the data base. In future we plan to audit compliance and patient satisfaction prospectively.

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P124

Accelerated renal impairment in a patient with type 2 diabetes with an inadequately investigated incidental adrenal adenoma

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A 54-year-old Asian man with type two diabetes diagnosed 6 years ago was admitted to hospital in May 2011 because of deteriorating renal function. His eGFR had been stable until April 2011 when his eGFR dropped from 37 in January 2011–2021. Repeat eGFR 2 weeks later was 17 and his family doctor felt he needed to be admitted to hospital. He was on metformin alone which was stopped. His HbA1c was 6.4% (48 mmol/mol). He had hypertension and was on four antihypertensive agents. He had no retinopathy and no peripheral neuropathy. As he was well he was discharged and was seen in our joint renal/diabetes clinic. His renal immunology was negative and he proceeded to a renal biopsy. This showed features of diabetic nephropathy and moderate chronic damage with associated arterio- and arteriolosclerosis. The number of viable glomeruli present was small and it was not possible to perform immunohistochemistry or electron microscopy. It was noted that he had an adrenal adenoma approximately one cm in diameter which was found incidentally in 1999. Renin and aldosterone levels were suggestive of primary hyperaldosteronism but this had not been followed up. He had a repeat scan in 2005 and the adrenal lesion had not increased in size after 6 years and was not followed up again. A repeat scan in July 2011 showed that there was minimal increase in size of the adrenal mass to 1.2 cm. Twenty-four hours urine collections excluded phaechromocytoma and Cushing's syndrome. Renin aldosterone ratio confirmed primary hyperaldosteronism. His eGFR had dropped further and he started haemodialysis. As it was planned for him to have a renal transplant he was referred for adrenalectomy which was carried out laparoscopically. Two cortical adrenal adenomas were excised. Appearances were consistent with Conn's syndrome. His blood pressure dropped and he came off all his antihypertensives.

Inadequate follow up/delay in investigating his incidental adrenal adenoma may have resulted in accelerated deterioration of his renal function.

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P125

Nephrogenic diabetes insipidus caused by lithium toxicity

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A 59-year-old gentleman with schizoaffective disorder, treated with long-term lithium therapy and depot fluphenazine, underwent elective cystectomy and ileal conduit formation for transitional cell bladder carcinoma.

Post operatively, he developed acute renal impairment, evidenced by a fall in eGFR from 68 to 26 ml/min per 1.73 m^2. This resulted in accumulation of lithium to a toxic level of 1.82 mmol/l (0.4–1.0); despite stopping lithium, serum sodium and urine output increased progressively and his fluid balance became negative. He became aggressive and his Glasgow coma scale (GCS) score fell to 12/15. By post-operative day 12, urine output was 12 l/24 h with a serum sodium of 168 mmol/l, plasma osmolality of 358 mOsm/kg and inappropriately dilute paired urine osmolality of 211 mOsm/kg.

The endocrinology team identified lithium toxicity as the cause of new onset nephrogenic diabetes insipidus. Intravenous fluid was administered to match urine output. Over the following week, as his lithium level fell to a sub-therapeutic level of 0.23 mmol/l, the patient's polyuria improved to 4.5 l daily and serum sodium fell to 160 mmol/l. Intravenous fluid was then reduced to match half his daily urine output, supplemented with oral fluids as his consciousness level normalised. Four weeks post-operatively, his serum sodium and eGFR were completely normal (137 mmol/l and 63 ml/min per 1.73 m^2 respectively). Urine output remained high at ~3.5 l/day, but he was able to match this with oral intake and maintain normal serum sodium. The psychiatry team advised that he no longer required lithium and he was discharged on depot fluphenazine alone for his mental health. A formal water deprivation test has been arranged, given that he continues to experience thirst and polyuria.

In summary, we present a case of acute nephrogenic diabetes insipidus, secondary to lithium toxicity that arose from early post-operative renal injury.

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P126

Title: A case of primary hypoadrenalism secondary to amyloidosis

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Introduction

Endocrinopathy is frequently seen in systemic amyloidosis and commonly involves thyroid and gonads. We report a case of primary adrenal failure secondary to systemic light chain amyloidosis (AL), involving kidney, liver, spleen, gut, nerves and tongue.

Case

A 42-year-old Somalian lady presented with 2-year history of lethargy, febrile episodes and 21 kg weight loss. Investigations showed increase in serum lambda light chain 103 mg/l (5.7–26.3), paraproteinaemia (7 g/l), 8% plasma cells on bone marrow aspirate and a diagnosis of lambda light chain secreting plasma cell dyscrasia was made. She subsequently developed renal failure and renal biopsy confirmed systemic amyloid light chain (AL) amyloidosis. CTDa chemotherapy (attenuated cyclophosphamide, thalidomide, dexamethasone) was initiated. She further developed macroglossia, peripheral and autonomic neuropathy and altered bowel habits. Amyloidosis was further confirmed on duodenal biopsy and serum amyloid protien (SAP) scintigraphy, demonstrating moderate total body amyloid load including liver and spleen.

Two years later, she was admitted following cardiac arrest from neutropaenic sepsis and renal failure requiring external cardio-pulmonary support. Hypotension did not resolve (mean BP: 76/44 mmHg) despite overall recovery through resolution of sepsis and renal failure (haemodialysis). Hypoadrenalism was clinically suspected and was confirmed through short synacthen test (60 min cortisol response: 128 nmol/l). Hypotension resolved (Mean BP:126/80 mmHg) on parenteral hydrocortisone (400 mg/day) and oral fludrocortisone (50 μg/day).

Progress

A normal pituitary scan and an appropriate pituitary response (IGF1 16.3 (13–37 nmol/l), LH <1 (2–12 IU), FSH <1 (2–10 IU), TSH 1.05 (0.35–6 mU/l), T_4 13.5 (9–26 pmol/l), ACTH 17.4 (<46.1 ng/l), prolactin 881 (<600 mu/l)) ruled out secondary hypoadrenalism. She is haemodialysis dependent and is currently stable on oral hydrocortisone (20 mg/day) and fludrocortisone (50 μg/day).

Conclusion

Diagnosis of adrenal insufficiency can be challenging in critically ill patients diagnosed with conditions involving multiple organs, such as systemic amyloidosis. High degree of clinical suspicion is needed to prevent this potentially fatal condition, as endocrine dysfunction in systemic amyloidosis is more frequent than it was once thought.

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P127

A challenging case of recurrent disabling severe hypoglycemic episodes

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Nesidioblastosis is a well recognized cause of persistent hyperinsulineamic hypoglycemia of infancy. Regardless of the pancreatectomy procedure used,

hypoglycaemia may recur during long term follow up. We describe a challenging case of recurrent hypoglycaemic episodes in a young adult.
We describe a 33-year-old landscape gardening laborer with history of insulin treated diabetes diagnosed at the age of 3 weeks old following subtotal pancreatectomy for nesidioblastosis. He presented with hypoglycemia and required partial pancreatectomy when he was 5 days old followed by subtotal pancreatectomy when 3 weeks old for nesidioblastosis. He was referred by his GP with 6 months of worsening hypoglycemia in spite of making appropriate insulin and dietary adjustments. He had four-five episodes of severe hypoglycemia nee ding to go to emergency department over this 6 month period and had loss of hypoglycemic awareness. On initial screening he had detectable C-peptide 0.12 μg/l (NR 1.1–4.4), insulin levels <0.5 mIU/l (NR 2.6–24.9). MRI and CT scan of abdomen showed a possible small area of residual pancreatic tissue in the region of head of pancreas. Octreotide scan did not show any increased uptake in this area.
Imaging has failed to show anything that was felt to surgically treatable. Octreotide scan showed no significant uptake reducing the potential for other therapeutic options at this stage. He is now taking Creon and a basal bolus insulin regimen with Novorapid and Detemir. He is currently adjusting his insulin and making life style changes in order to reduce hypoglycemic episodes. His hypoglycaemia is continuing to improve and he has returned to work.

- Should we do anything else at this stage to address his unexplained hypoglycaemic episodes?
- How do we control his hypoglycaemia if this gets worse in the future? Should total pancreatectomy be considered as an option to prevent disabling hypoglycaemia in future?

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P128

'Gastroparesis' in a patient with uncontrolled diabetes: NOT always autonomic neuropathy!

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Case: 81-year-old male with history of insulin-dependent diabetes mellitus (diagnosed in 1984, HbA1c of 8.9%), hypertension presents to the emergency department with 6 days of worsening nausea, vomiting and abdominal discomfort to the point where he couldn't tolerate any oral diet. He was admitted thrice in the last year with similar complaints. A routine evaluation was always done including X-ray, routine endoscopy which showed gastroparesis and no abnormal cytology in brushings. He responded to motility agents after a few days and was discharged home with pro-kinetic agents. He was scheduled to receive a gastroenterology evaluation for pacemaker placement in few months. Now patient also complained of a 20 lb weight loss, heart burn and early satiety, which he thinks are new for him. An abdominal X-ray showed gastric outlet obstruction. The gastroenterologist repeated an endoscopy which showed narrowed gastric outlet, mild erythema of the gastric and esophageal mucosa. A high index of suspicion lead to a endoscopic ultrasound-guided biopsies showing a diffusely thickened wall of the gastric antrum that came back positive for adenocarcinoma. The anatomic pattern confirmed the diagnosis of Linitis plastica. This was the reason for the patients gastroparesis. A PET scan confirmed FDG uptake diffusely in the stomach wall, small lesion in his liver and a few nodes.
Discussion
Diabetes-induced gastroparesis is seen in 5–12% of all diabetics. It is postulated to be secondary to the autonomic neuropathy involving the vagus nerve. It progressively gets worse with no definitive solution other than the novel treatment of using Gastric Pacemaker which has been shown to have limited success. The complacency and questionable benefits of the pacemaker placement makes many of us not do a thorough work up for such a patient, which might lead to major under diagnosis of these life-threatening conditions.

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P129

Audit on continuous subcutaneous insulin infusion

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Insulin pump is one of the treatment options for patients with type 1 diabetes mellitus. In July 2008, NICE has issued Technological Appraisal (TA 151) regarding insulin pump therapy guidance.

Objective of the audit
To measure current practice in Colchester General Hospital in continuous subcutaneous insulin infusion – CSII (insulin pump) for the treatment of diabetes mellitus against the recommendations in the technological appraisal (TA 151).
Methodology
Review of patients' notes.
Summary of findings

- Mean age of the patients is 43.1 (range 18–73) years.
- 27 females and 14 males patients are on CSII.
- The average duration of diabetes in these 41 patients is 25.7 (range 5–47) years.
- The average duration on CSII is 3.15 (range 1–7) years.
- Reason for starting on CSII – 25 patients with hyperglycaemia, 12 patients with hypoglycaemia and four patients with both reasons.
- CSII was initiated in all patients (100%) by a trained specialist team.
- The specialist team provided structured education programme and advice on diet, lifestyle and exercise appropriate for people using CSII in all patients (100%).
- 27 patients (66%) have improved glycaemic control ($>0.5\%$ HbA1c reduction) 1 year after CSII.
- Six patients (43%) out of 14 patients assessed have reduced number of hypoglycaemia or improved in hypoglycaemia awareness.
- Twenty patients (49%) have lost weight 1 year after CSII.

Conclusion
Overall, the current practice in Colchester General Hospital in CSII therapy (insulin pump) for the treatment of diabetes mellitus is in line with the recommendations set by NICE's TA 151.

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P130

Radioactive iodine-induced hyperparathyroidism

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Case history
A 27 years old lady having presented with a neck mass, underwent a total thyroidectomy for thyroid malignancy in 1967. Histology confirmed papillary carcinoma of the thyroid. In 1968, she complained of tiredness and mild neck swelling despite TSH suppressive therapy. Her thyroid uptake study showed a residuum of thyroid tissue within the neck. She went on to have radioiodine ablation therapy on two separate occasions. She responded well with no evidence of residual tumour. She was maintained on TSH suppressive therapy thereafter with levothyroxine.
During the follow up, at the age of 72 years she began to complain of low energy level, constipation and tiredness which warranted further investigations.
Investigations:
Blood results normal FBC, U&E, LFT, vitamin D and elevated calcium of 2.74 mmol/l and PTH of 10.1 (normal 2.5–7.6 pmol/l) pmol/l. DXA showed spine L1–L4, *T*-score -1.1, and left hip total *T*-score -1.7. Renal ultrasound scan showed no evidence of nephrocalcinosis.
Results and treatment
Biochemical profile was consistent with primary hyperparathyroidism.
Following discussion, the patient opted for conservative management.
Conclusion and point of discussion:
External beam radiation (5–30 Gy) is associated with a dose-dependent occurrence of primary hyperparathyroidism, following a latency period of 20–45 years. In contrast, radioiodine therapy delivers a local dose of radioactivity to the thyroid and adjacent parathyroid glands in the region of 50–100 Gy for benign disease. Radioiodine has been associated with an increased risk of hypoparathyroidism.
We hypothesise that our patient developed hyperparathyroidism as a consequence of her previous radioiodine therapy, the delivered dose to the parathyroids being relatively lower due to only a small amount of residual thyroid tissue at time of ablation. This case highlights the importance of being aware of to the long-term sequelea of radioiodine therapy and the need for continued observation.

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P131

A case of phaeochromocytoma presenting as incidentloma

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A 83-year-old gentleman admitted with nausea and feeling generally unwell. Past medical history of hypertension, duodenal ulcer, previous gastric surgery, polymylagia rheumatica and type two diabetes mellitus. Chest XR showed right basal pneumonia which was treated with antibiotics. Patient admitted to significant weight loss therefore he had thoracic-abdominal CT scan performed which showed 4 cm by 4 cm solid cystic lesion in the left adrenal gland possibly malignant. Subsequently magnetic resonance imaging scan revealed left adrenal heterogeneous lesion which could be either primary or metastatic. 24 h urinary metadrenaline 7.2 μmol/24 h, normetadrenaline 4.1 μmol/24 h. These findings were consistent with diagnosis of phaeochromocytoma. He had experienced hypotensive episodes associated with dizziness these were treated with intravenous fluids to expand his intravascular volume. He did not tolerate phenoxybenzamine because of dizziness and low blood pressure. He was assessed for surgical treatment to his phaeochromocytoma but in view of his multiple co-morbidity he declined surgery therefore he was managed conservatively. For nearly 2 years he remains under outpatient follow-up and his repeated imaging showed slight enlargement of his original lesion but no new lesion.

Most common symptoms of phaeochromocytoma are headaches, palpitations, sweating and tachycardia. Up to 8% of patients may be asymptomatic and are discovered incidentally on imaging done for unrelated symptoms as in our case. In the elderly decrease in baroreceptor function with age as well as concomitant disease, signs and symptoms of which can confound the paeochromocytoma diagnosis. This case clearly highlights management challenges of phaeochromocytoma in the elderly with complex co-morbidity.

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P132

Propylthiouracil-induced severe agranulocytosis!

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Case

A 43 years old female with a PMHx of Graves disease initially treated with methimazole (discontinued due to arthralgias) and started on propylthiouracil (PTU). Three years ago she went into remission and the PTU was stopped. About 6 weeks ago, the patient started experiencing typical symptoms of hyperthyroidism including palpitations and hot sweats so she was restarted her on PTU. Patient started noticing fever, chills, myalgias and dry cough. In the hospital her Temperature was 102.4 F, pulse was 137 beats/min with sinus tachycardia. Labs showed Wbc – 1.2 K/cm with neutrophils of 7%, TSH – 0.2 μIU/ml, T_4 – 3.2 ng/dl and T_3 uptake – 42%. Peripheral smear showed decreased white blood cell count with otherwise mature lymphocytes and RBC. Physical examination showed hyperreflexia of the extremities with tremors. She was started on antibiotics for neutropenic fever and propranalol for the sinus tachycardia. An extensive work up for neutropenia was negative including vitamin B12, folic acid, serology for viral infections (hepatitis, HIV, CMV, EBV, parvovirus), lupus antigen and pan-cultures. Bone marrow aspirate revealed maturation arrest in granulocytic lineages at the myelocyte/metamyelocyte stage. Flow cytometry showed 4% myeloblasts and very few normal maturing myeloid cells. PTU was thought to be the culprit as a diagnosis of exclusion. Patient was given granulocyte stimulating factor and this improved her WBC count.

Discussion

Agranulocytosis is a rare but serious complication of thionamide therapy, with a prevalence of 0.1–0.5%. It can occur as early as 10 days after starting therapy or may be delayed for up to 3 months. The immune reactions occur via IgE-mediated hypersensitivity, drug-induced IgG/IgM response and neutrophil–drug complexes. Antigranulocyte antibodies have been reported in some patients which could explain the reaction for the second time initiation of the medication.

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P133

Rare onset of polyendocrinopathies in a pediatric patient

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We present this case of a 7-year-old female who had been well, without fever, polyuria or polydipsia, nor temperature intolerance. There was no known family history of auto-immune disorders. Physical exam was unremarkable but further labs revealed hyperglycemia (329 mg/dl), venous pH 7.37, glycosuria (no ketonuria). Her hyperglycemia resolved with intravenous and then subcutaneous insulin. Further labs revealed glutamic acid decarboxylase antibodies to be high (>30.0), and HbA1c 8.9%. Interestingly, she was also noted to have a decreased TSH (<0.008 μIU/ml) and elevated thyroid peroxidase antibodies (>600 U/ml). She was diagnosed as a new onset type 1 diabetes and Grave's disease and started on an insulin regimen with methimazole daily.

Grave's disease is the most common cause of hyperthyroidism in children and adults. While type 1 diabetes also presents in childhood, both conditions presenting coincidentally is extremely rare and uniquely categorized as a polyglandular autoimmune syndrome (PAS). There are three types of PAS; type 3 is when autoimmune thyroiditis occurs with another organ-specific autoimmune disease, 3A being with diabetes mellitus. This condition, associated with HLA type 2, has been noted to have an autosomal dominant pattern of inheritance with incomplete penetrance, usually occurring after the age of 30. Patients with PAS III must undergo lifelong monitoring of hormones and/or vitamin replacement therapy to avoid the development of new glandular failures.

Management of this condition in the pediatric population is challenging primarily because of the impact on families who have to learn to manage multiple conditions. Moreover, the pediatric age group may be asymptomatic, or have symptoms but remain unnoticed for a long time, putting them at risk for chronic lymphocyte infiltration. This case proves an important teaching point to routinely screen pediatric patients for autoimmune abnormalities in multiple organ systems because autoimmune endocrinopathy can be insidious in onset and necessitates adequate surveillance.

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P134

Diarrhoea and an adrenal incidentaloma

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A 53-year-old lady of African origin presented to the emergency department with a 2 months history of watery diarrhoea associated with anorexia, general deterioration and weakness. She had lost 10 kg over 2 months. There was no history of recent travel abroad. Her past medical history included a cardiac arrest in 2000 (hypertrophic cardiomyopathy) and she had an internal defibrillator implanted for Brugada-like syndrome. She had hypertension since 1999 and it was noted that she was taking five different antihypertensives (olmesartan + amlodipine, spironolactone + altizide, moxonidine) and she was also on sotalol. On further questioning she also admitted to having hot flushes which she had attributed to the menopause. She had had an admission under the gastroenterologists 2 weeks previously and had a 24 h urine collection which had shown normal 5HIAA excretion however her chromogranin A was found to be elevated at 294 ng/ml (NR <100) and an octreotide scan had been requested which subsequently came back showing a chain of nodules overexpressing somatostatin receptors (subtype two and five) in the left para-aortic retroperitoneal region (in front of the left renal hilum). VIP and gastrin levels were normal. CT scan of the thorax was unremarkable. CT scan of the abdomen/pelvis showed no focal abnormality in the liver. Multiple mesenteric infra-centrimetric ganglions were seen. A 3.2 cm left adrenal mass was noted and there was also a right ovarian cyst. Three further urine collections were requested and in one collection the 5HIAA levels were elevated at 22.4 ng/24 h (NR <8). Catecholamine levels were normal.

It was planned for her to have a laparoscopic biopsy of one of the nodules noted on the octretide scan. However, at laparoscopy the surgeon could not visualize the nodules and removed the left adrenal mass. Subsequently the aldosterone level came back at 8.9 ng/dl (NR 4–20) and her renin level was <0.5 μUI/ml (NR 2.8–39.9), results compatible with a diagnosis of primary hyperaldosteronism. She came off all her anti-hypertensives. Unfortunately following her operation she developed a deep vein thrombosis and further investigations to look for the source of her carcinoid syndrome are on hold.

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P135

Multiple autoimmune diseases: is it rare?

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A 56 year old female who was referred to the Endocrine clinic with a history of progressive anterior neck swelling, weight gain, memory impairment and sluggishness. On examination she was found to be pale, had an anterior neck mass which moved with swallowing but not with tongue protrusion, is diffuse, firm non tender, measuring 8 × 6 cm. There was associated submandibular lymph node enlargement.

Her thyroid function test revealed primary hypothyroidism (FT_3 2.5 pmol/l, FT_4 2.7 pmol/l and sTSH 8.01 μIU/l) and thyroid autoantibodies were markedly elevated (TPOAb 55.5 IU/ml, TgAb 197.3 IU/ml). Her ESR was also markedly elevated (131 mm/h westergren method). A diagnosis of Autoimmune hypothyroidism was made and patient was commenced on L-thyroxine.

6 weeks later she complained of dry mouth and mouth ulcers and further questioning revealed that she had recurrent joint pain and swelling especially of the small joints of the hands, hypopigmentation of the skin and the need to drink water to swallow food. On examination she had bilateral parotid fullness, dry tongue, hypopigmented patches on the hands and swelling of both proximal and distal interphalangeal joints. Spit test was about 1 ml.

LE cells was positive and Antinuclear antibody was positive with homogenous pattern.

Protein excretion in 24 h was 1.2 g end patient was referred to the Nephrologist. The following diagnoses were considered; autoimmune hypothyroidism, systemic lupus erythematosus with nephritis and Sjogren syndrome. She was subsequently treated with L-thyroxine, prednisolone and cyclophosphamide and is presently on follow up.

Conclusion

Autoimmune diseases are not uncommon in older women. The presence of one warrants an intense search for others.

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Cytokines and growth factors

P136

Effect of acute hypoxia upon myostatin expression in healthy individuals

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We previously showed acute hypoxic conditions result in atrophy of myotubes *in vitro*. Chronic hypoxic exposure *in vivo* induces muscular atrophy in healthy mountaineering individuals and patients with COPD. Myotubes *in vitro* increase myostatin expression in response to hypoxic exposure. Further, hypoxic COPD patients show cachexia and increased serum myostatin expression. However, *in vivo* results are cofounded by disease factors in COPD patients or environmental factors in mountaineers. We therefore hypothesize that healthy normal individuals exposed to hypoxia will increase expression of serum and intramuscular myostatin protein. We exposed eight healthy males to 2 h of hypoxia (12% O_2) or control condition (21.93% O_2) in a cross-over design, separated by 14 days. Muscle biopsies and arterialised blood samples were taken pre-hypoxia ($t=0$), immediately post-hypoxia ($t=120$), and 3.3 h post-hypoxia ($t=320$). Plasma was analysed for total myostatin using a commercial ELISA. Bioactive myostatin peptide levels from biopsies was visualised by western blot in a standard manner.

Hypoxia successfully perturbed homeostasis, with saturation of capillary O_2 decreased across all time-points, increased heart rate during hypoxia, and trends towards increased LLAMS scores under hypoxic conditions. Analysed biopsies suggest a decrease in intramuscular myostatin at $t=320$ coupled with an increase in plasma myostatin concentration at $t=320$ as measured by plasma ELISA.

Hypoxic exposure is associated with myotube atrophy *in vitro* and muscle atrophy *in vivo*. Myostatin is an anti-anabolic protein that may underlie atrophy under hypoxic conditions. We suggest hypoxia stimulates muscle release of myostatin into the circulation where it has systemic effects. Further, if myostatin signalling is altered in healthy individuals during acute hypoxic exposure, this may explain the muscle atrophy seen in mountaineering and in diseases such as COPD.

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P137

Genes for IGF2 and related IGF binding proteins are associated with longitudinal trends in BMI

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High IGF2 has been associated with longitudinal weight loss. We wished to study associations of genes coding for IGF2 and for binding proteins that have preferential IGF2 affinity (IGFBP2, IGFBP5 and IGFBP6) in 991 Caucasian subjects from Salford with type 2 diabetes.

Fifteen *IGF2*, four *IGFBP2*, eight *IGFBP5* and one *IGFBP6* SNPs were successfully genotyped. Longitudinal BMI data for the years 2002 to 2009 was obtained from integrated primary care and hospital electronic medical records. Mixed effects regression analyses were used to study SNPs as predictors of longitudinal BMI in models adjusted for age, gender and prescription of common diabetes medications that could affect weight (metformin, sulphonylureas, thiazolidinediones and insulin).

IGF2 rs12417332 and *IGFBP5* rs4674107 was associated with weight gain, while *IGFBP2* rs7603372, *IGFBP2* rs9341105, *IGFBP5* rs741384 and *IGFBP5* rs7426116 were associated with longitudinal weight loss. When all the significantly associated proteins were taken together in a stepwise regression model along with age, gender and the earlier medications as covariates, associations for three SNPs – *IGFBP2* rs9341105 ($\beta=-0.12$, 95% CI -0.20 to -0.04, $P=0.001$), *IGFBP5* rs741384 ($\beta=0.12$, 95% CI 0.06 to 0.18, $P<0.001$) and *IGF2* rs12417332 ($\beta=-0.12$, 95% CI -0.22 to -0.023, $P=0.016$) remained.

IGF2 has been previously reported to have longitudinal associations with weight change, and the biding proteins IGFBP2 and IGFBP5 can potentially modify IGF bioavailability. This study suggest that gene variations for all these proteins can partly determine longitudinal weight trends in diabetes.

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P138

Comparing the effect of rimonabant and metformin on vascular endothelial growth factor levels in women with PCOS

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Background

PCOS is associated with a clustering of cardiovascular risk factors. Increased serum levels of vascular endothelial growth factor (VEGF) are thought to be proatherogenic, and have also been found to be elevated in PCOS. This study was undertaken to determine the changes of VEGF in patients with PCOS after rimonabant and/or metformin intervention.

Methods

A 6-month randomised open labelled parallel study of rimonabant or metformin in 20 patients with PCOS with a body mass index ≥ 30 kg/m^2. Subsequently, patients who were on 3 months of rimonabant were changed over to metformin for 3 months, whereas those on 3 months of metformin were continued on metformin for another 3 months.

Results

After 3 months of rimonabant (V1/2) there was a significant increase in VEGF (99.24 + 17.61 vs 116.16 + 15.82 pg/ml, $P< 0.01$) whilst there was no significant change in VEGF in the metformin group (110.29 + 25.17 vs 111.47 + 24.84 pg/ml, $P=0.75$). However there was a significant decrease in VEGF in the metformin group between month 3 and month 6 (V2/3) (111.47 + 24.84 vs 91.21 + 16.44 pg/ml, $P=0.04$). There was also no significant change in VEGF during metformin treatment in the rimonabant group (V2/V3) (116.16 + 15.82 vs 106.45 + 36.43 pg/ml, $P=0.18$).

Weight change in the rimonabant group was V1/V2 104.6 + 4.6 vs 98.4 + 4.7 kg, $P<0.01$; V2/V3 98.4 + 4.7 vs 98.6 + 4.8 kg; $P=0.96$; in the metformin group V1/V2 103.8 + 3.9 vs 102.2 + 4.1 kg, $P=0.08$; V2/V3 102.2 + 4.1 vs 100.9 + 4.2 kg, $P=0.02$. There was no correlation between changes in weight and VEGF levels.

Conclusion
Metformin decreased VEGF levels after 6 months independent of weight change, however rimonabant increased VEGF levels despite significant weight loss suggesting a proatherogenic pharmacological effect.

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P139

IGF binding protein-2 is associated with all-cause and cardiovascular mortality in type 2 diabetes

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Low IGFBP2 is associated with the metabolic syndrome, but high IGFBP2 is longitudinally associated with worsening renal function. IGFBP2 binds IGF2 (and IGF1) and modified IGF bioavailablilty. The aim of our study was to identify whether baseline IGFBP2 concentrations were associated with all-cause and cardiovascular mortality in type 2 diabetes.
554 subjects (59.3% male, mean age 63.7 (S.D. 10.7)) with type 2 diabetes from the Salford Diabetes cohort were studied. IGFBP2 as well as IGF1, IGF2, IGFBP-one and IGFBP-three were measured once-in samples withdrawn at baseline in 2002-2003. Clinical data regarding these 554 subjects were then followed up for death until August 2011.
Clinical data was obtained from electronic records and death data from Office of National Statistics. 132 deaths were recorded in the study population, with cause of death available in 124 of them. Main causes of death were – 51 cardiovascular deaths (including myocardial infarction (19), stroke (7), heart failure (6), others (9); cancer (35); Sepsis (15), COPD (6), renal failure (2).
Cox regression analyses done separately for each IGF protein, age, baseline cardiovascular variables and diabetes duration, log rank assessments done for gender, previous history of MI or stroke. Positively associated variables studied in Cox-proportional hazard regression model after satisfying proportionality assessments.
60% of deceased were male. High baseline IGFBP2 wasignificantly associated with higher all-cause mortality (hazard ratio 1.001, 95% CI 1.000–1.003, $P=0.005$). IGF1 (HR 1.003, 95% CI 1.00001–1.007, $P=0.044$) and IGFBP2 (HR 1.004, 95% CI 1.002–1.007, $P=0.001$) were significantly associated with cardiovascular mortality.
IGFBP2 is predictor of all-cause and cardiovascular mortality. The nature of the association remains to be clarified, it may be related to decreased IGF2 bioavailability and increased frailty.

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Growth and development

P140

Comprehensive spatio-temporal expression profiling reveals a complete natriuretic peptide system in the developing Zebrafish (*Danio rerio*)

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C-type natriuretic peptide (CNP) is the ancestral and most highly conserved member of the mammalian natriuretic peptide family. Although broadly expressed throughout the periphery (bone, gonads, kidneys), CNP is the major natriuretic peptide of the brain and CNS and is suspected to influence neuroendocrine function. Our recent studies using fetal human pituitary samples has shown that the gene encoding CNP, *NPPC*, is expressed early on in pituitary development but a comprehensive understanding of CNP expression *in utero* remains to be established. Employing the versatile *Danio rerio* (Zebrafish) as an excellent vertebrate model of development, we sought to establish the spatio-temporal expression of components of the natriuretic peptide system throughout development. Zebrafish embryos were captured at time points ranging from three hpf up to 120 hpf prior to extraction of total RNA. Additionally, adult Zebrafish were euthanized and total RNA was extracted from both head and body separately. RT-PCR enabled us to screen for the expression of *nppa* (ANP), *nppb* (BNP), *nppc2, cnp3, nppc4, nppcl* (CNPs), *npr1a, nprA* (GC-A), *nprB* (GC-B) *and nprC* (clearance receptor gene). All these genes were expressed, but clear temporal differences between the four distinct CNP transcripts were observed. Digoxigenin-labelled RNA probes were synthesised from all cloned PCR products prior to *in situ* hybridization using embryos captured at 24, 48, 72, 96 and 120 hpf. The four CNP genes each demonstrated individual spatial expression, yet were all concentrated in the head and notochord during development with the GC-B receptor *nprb*, more restricted in its expression. *In silico* phylogenetic analysis of the four CNP genes demonstrated evolutionary conservation was maintained between these transcripts in Zebrafish and conserved across vertebrate species. These data reveal the presence of an intact natriuretic peptide system in developing Zebrafish, and will facilitate a reverse genetics approach to identify their functional roles within neuroendocrine development.

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P141

Impaired heart function and cardiac maturation in fetal mice with disrupted GR signalling in vascular smooth muscle and cardiomyocytes

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Glucocorticoid signalling is essential for cardiac maturation late gestation. In mice, global glucocorticoid receptor deficiency ($GR^{-/-}$) severely impairs cardiac function and ultrastructure at embryonic day (E) 17.5. To dissect direct effects of GR deficiency in the heart from effects on other systems, Sm22α-Cre mice were crossed with 'floxed' GR mice to generate SMGRKO mice with disrupted GR signalling in cardiomyocytes and vascular smooth muscle cells. GR mRNA and protein levels in hearts of fetal SMGRKO mice are reduced by ~65%, without any change in other organs (liver, kidney, lung) or in plasma corticosterone levels.
In contrast to global GR-deficiency, late gestation SMGRKO mice are viable but still show oedema. In vivo ultrasound analysis showed impaired heart function with elevated myocardial performance index (MPI) in SMGRKO compared to floxed littermate controls (SMGRKO 0.43 ± 0.01 vs control 0.49 ± 0.2, $n=20$–26, $P<0.05$). Histopatholgy showed cardiomyocytes in SMGRKO fetuses are irregularly shaped and smaller than in controls, and fail to align in the outermost layer of the compact myocardium. Electron microscopy revealed immature and disorganised myofibrils in SMGRKO cardiomyocytes, similar to global GR-deficiency. SMGRKO hearts showed mRNA changes indicating reduced contraction (Myosin Heavy Chain-α), energy metabolism (hexokinase-1, PGC-1α) and calcium handling (Cav1.2, RyR2, SERCA2a) in SMGRKO at E17.5.
Thus, the cardiac phenotype of SMGRKO fetuses is strikingly similar to that of global GR-deficienct, albeit of lesser magnitude owing perhaps to incomplete GR deletion in the heart. However, in contrast to the smaller hearts of globally GR-deficient fetuses, hearts of E17.5 SMGRKO mice are normally sized. This suggests that smaller hearts in global GR-deficiency is secondary to lack of GR elsewhere.
These data support a role for GR within cardiomyocytes in promotion of cardiac maturation.

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P142

Impact of a low protein diet during pregnancy in sheep on insulin and vascular endothelial growth factor signalling

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Introduction
Epidemiological evidence suggests that a poor diet during pregnancy leads to offspring with higher risk of chronic diseases in adulthood. Our previous work in sheep showed that mild protein-energy malnutrition in early gestation results in decreased insulin signalling in the liver, and changes in vascular endothelial growth factor (VEGF) in the kidney. The current study explored whether these changes are generalised phenomena, or organ-specific.
Methods
Pregnant ewes were fed either a control diet providing adequate dietary protein (control, C; $n=15$), or a low protein diet (50% protein restriction) during early pregnancy (0–65 days of a 147 day gestation; low protein, LP, $n=16$). Fetuses were killed at day 65 gestation, or carried to term and killed in adulthood at two

years of age. Organs were snap frozen in liquid nitrogen, or preserved in 4% paraformaldehyde. The mRNA was measured using quantitative PCR, and protein levels assessed using immunohistochemical techniques.
Results
In the fetal kidney VEGF mRNA and protein was reduced by 30–40% in LP vs controls, but as adults the reverse was true. In contrast, in the fetal liver, VEGF mRNA was increased threefold in LP fetuses vs controls, and was not different between groups in the adults. However, as adults, the LP exposed animals had hepatic insulin resistance, and down-regulated insulin-sensitive genes. In the adult kidneys, at this stage there were no significant reductions in the insulin pathway genes in the LP group.
Conclusions
A low protein diet leads to hepatic insulin resistance and effects on VEGF in the kidney. However, these effects are organ-specific and do not translate to other organs.
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P143

Reduced glucocorticoid action in obese pregnancy associates with increased birth weight and macrosomia

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Background
One in five women is obese at antenatal booking. Maternal obesity increases risk of offspring complications including higher birthweight. We hypothesised that this is mediated by altered action of maternal glucocorticoids, key regulators of fetal growth and development. We compared cortisol levels during pregnancy and placental glucocorticoid sensitivity in obese and lean women.
Methods
With ethical approval serum cortisol levels were measured at 16, 28 and 36 weeks gestation in $n=173$ class III obese (BMI 44.0 ± 4.5 kg/m^2) and $n=107$ lean (BMI 22.8 ± 1.6 kg/m^2) pregnant women. Serial corticosteroid binding globulin (CBG) concentrations were measured in a subset ($n=39$ lean, 26 obese) and free cortisol levels calculated using Coolen's equation. Salivary cortisol was measured at bed-time, waking and 30 mins after waking. 11β-hydroxysteroid dehydrogenase type 2 (*11βHSD2*), which inactivates cortisol, and glucocorticoid receptor (*GR*) mRNAs were measured in first trimester ($n=34$) and term ($n=56$) placental samples.
Results
Cortisol levels rose similarly during pregnancy in obese and lean, but were significantly lower throughout pregnancy in obese women ($P<0.05$). The diurnal rhythm was maintained. CBG levels also increased, though change was lower in obese (1.21-fold (±0.32) vs 1.56-fold (±0.38), $P<0.01$). In obese, lower calculated free cortisol at 16 weeks gestation was associated with higher birth-weight after adjustment for confounders ($r=-0.46$, $P<0.05$). Placental expression of *11βHSD2* increased in association with increasing obesity in early pregnancy ($r=0.46$, $P<0.01$) and was highest in term placentas in obese women with macrosomic (>4000 g) offspring ($P<0.05$). Placental expression of *GR* also increased in association with increasing obesity in early pregnancy ($r=0.45$, $P<0.01$), but was lowest in term placenta from obese women with macrosomic offspring ($P<0.05$).
Conclusions
Lower circulating and bioavailable cortisol levels in early obese pregnancy, together with a greater placental barrier to maternal glucocorticoids may contribute to higher birth weight and macrosomia in offspring of obese women.
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P144

Identification of twenty-two novel GATA3 mutations in hypoparathyroidism-deafness-renal dysplasia syndrome

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The hypoparathyroidism-deafness-renal dysplasia (HDR) syndrome is an autosomal dominant disorder caused by germline mutations of the dual zinc-finger (ZnF) transcription factor, GATA3. To date, 51 *GATA3* mutations have been reported, which can be divided broadly into three structural-functional classes: i) mutations that lead to a loss of DNA binding and involve ZnF2; ii) mutations, usually of ZnF1, that bind DNA but result in reduced DNA binding affinity; and iii) mutations, mostly of ZnF1, that do not alter DNA binding or affinity but lose protein interaction. To gain further insights into the structural-functional consequences of GATA3 mutations, we investigated 31 HDR patients for *GATA3* mutations. Venous blood was obtained after informed consent, as approved by the local ethical committee, and leukocyte DNA extracted. *GATA3* specific primers were used for PCR amplification and the DNA sequence determined. Twenty-two germline heterozygous *GATA3* mutations were identified, consisting of: nine missense mutations, four nonsense mutations, four frameshifting deletions, two frameshifting insertions and three splice site mutations. To further elucidate the molecular mechanisms altered by the mutants we functionally characterised six novel missense mutations: Arg299Gln, located in the linker region between ZnF1 and ZnF2; Thr326Ile, Arg330Trp, Ala341Asp, and Cys342Tyr, within ZnF2; and Tyr345Cys, located C-terminal to ZnF2. Investigation of nuclear localisation, DNA binding and gene transactivation revealed that: Thr326Ile resulted in loss of nuclear localisation; all the other mutations resulted in complete loss of DNA binding and reduction of transactivational activity by >90%, with the exception of Arg299Gln which led to decreased DNA binding and a >30% reduction of transactivational activity. Thus, our studies have identified an additional 22 germline *GATA3* mutations, associated with HDR, including one that defines a fourth class of GATA3 mutation, which is located in the ZnF1-ZnF2 linker region, and has an intermediate effect on DNA binding and gene transactivation.
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P145

Familial constitutional delay in growth and puberty is a condition with significant genetic heterogeneity and limited overlap with the timing of puberty in the general population

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Background
Pubertal timing has importance both for the individual, but also for public health. Previous studies estimate that 60–80% of variation in pubertal onset is genetically determined. Recently, a large genome-wide association study (GWAS) meta-analysis identified 42 loci for age-at-menarche (AAM), which explained 3.6–6.1% of the variation in the general population, but causal genes have not been identified.
CDGP is defined as pubertal onset at more than 2.0 standard deviations later than mean population age. CDGP therefore represents an extreme variant of normal pubertal timing and has repeatedly been shown to cluster in families, but the genetic factors behind CDGP remain elusive.
Aims
We hypothesise that in CDGP only a few genetic variants have a strong impact on the timing of puberty, and we aim to identify these using whole exome sequencing (WES).
Methods/Results
We selected seven very informative CDGP families who have been accurately phenotyped through long-term growth data. Filtering and annotation of over 2 million variants returned from WES of 52 individuals produced an extensive list of potential causative variants that segregate with the trait. No common gene mutations were apparent across the seven families, and pathway analysis did not identify common pathways across the families.
To examine for genetic overlap between causal genes in CDGP, and AAM in the normal population, we identified all genes in linkage disequilibrium with the GWAS loci with inclusive limits ($D'>0.8$, r^2 no limit, altogether 297 genes). Nine variants were seen in these 297 genes in our CDGP patients, all known non-synonymous single nucleotide polymorphisms. Sequencing of the nine genes in a further 288 CDGP individuals is currently underway for validation.
Discussion
Our results highlight the significant genetic heterogeneity in CDGP, and suggest limited overlap between the genetic factors regulating pubertal timing in the normal population and CDGP.

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P146

Pre-clinical investigation of therapy for segmental overgrowth caused by constitutive activation of phosphoinositide-3 kinas: lessons for cancer therapy

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Introduction
We recently reported cases of segmental overgrowth due to mosaic heterozygous activating mutations in the p110α catalytic subunit of PI3K. The index case presented with life-long, massive overgrowth of both legs with a lean upper body. Mobility was threatened by continued growth. mTORC1 inhibition has been effective at slowing excess growth due to loss of PTEN function, a negative regulator of PI3K. We hypothesised that mTORC1 inhibition would also be effective in this setting.
Methods/Results
Fibroblasts grown from a leg biopsy were confirmed to have a 50% burden of the p.*PIK3CA*.His1047Leu mutation, with higher basal and stimulated PIP3 levels and consequent hyperactivation of downstream PI3K-AKT signalling. Treatment of cells with everolimus 5 nmol/l for 120 h reduced basal and insulin-stimulated phosphorylation of AKT^{ser473} and p70S6K, however baseline PIP3 levels were twofold higher with everolimus treatment, this increase being amplified by insulin or EGF.
Discussion
This study highlights that chronic mTORC1 blockade leads to increased PIP3 levels with potential to enhance cytokinesis despite reduced AKT activation. We conclude that sirolimus, an mTORC1 inhibitor, may be effective in this patient, but careful monitoring for malignancy will be required. Our findings further underline concerns that cancers, especially those possessing activating PI3K mutations, could behave adversely with mTORC1-inhibitor monotherapy.
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P147

Effects of the endocrine disrupting herbicide, Atrazine, on pituitary development, gene expression and signalling pathways in Zebrafish (*Danio rerio*) and mouse pituitary cell lines

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Atrazine (ATR) is a widely used herbicide, with known effects as an endocrine disrupting chemical. Several studies have implicated ATR in causing disorders of sex development in reptiles, and chronic exposure can cause an increase incidence of intersex in fish, suggesting that the hypothalamo-pituitary–gonadal axis is a major site of ATR action. In this study, we investigated whether acute exposure (hours to days) to ATR could cause abnormalities in the development of Zebrafish larvae. Wild-type AB zebrafish larvae were exposed to ATR (1–10 μM) within 2 h post-fertilisation (hpf), and examined at 24, 48 and 72 hpf. Morphometric analyses revealed that ATR caused a concentration-dependent increase in pericardial oedema and hatching rates ($***P<0.001$) compared with DMSO-treated controls, but significantly reduced eye diameter ($**P<0.01$). In addition, ATR exposure caused a modest, but highly significant reduction in body length ($***P<0.001$). Subsequent *in situ* hybridisation analyses of these larvae at each of the indicated time-points revealed altered expression of pomc, prl and l h in ATR-treated Zebrafish. To establish whether the growth alterations observed in Zebrafish were also observed in pituitary cell lines, crystal violet assays were performed on αT3-1, LβT2 (gonadotroph) and GH3 (somatotroph) cells. ATR exposure for up to 72 h failed to significantly alter cell proliferation in any of the cell lines examined. RT-PCR analyses of pituitary gene expression in αT3-1 and LβT2 cells revealed that ATR enhanced expression of Egr1. However, the expression of Nr5a1 was inhibited in αT3-1 cells, but enhanced in LβT2. Finally, as ATR is thought to interact with phosphodiesterase enzymes, we examined the effect of ATR on cAMP accumulation in αT3-1 and LβT2 cells. Surprisingly, ATR failed to significantly alter cAMP in either cell line, whereas the diterpine, Forskolin, dramatically enhanced cAMP in both cell types ($***P<0.001$). These data reveal that ATR may have rapidly acting effects during development in Zebrafish, and that disruption to pituitary gene expression in particular could contribute to endocrine disorders in later life.
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P148

The spectrum of associated congenital anomalies in disorders of sex development: a review of the I-DSD Registry

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Background
Improved knowledge of the range of anomalies encountered in DSD may improve our understanding of the underlying aetiology. However, given the rarity of these conditions, thorough analysis of congenital anomalies in DSD has not previously been possible.
Aims
To discover the frequency of congenital anomalies in DSD, and to identify patterns of anomalies within specific conditions.
Methods
1050 registered cases on The I-DSD Registry (UKCRN#12729), currently used by 20 clinical centres in 14 countries, were examined. 649 (62%) had consent level to allow sharing suitable information. Case details were obtained from the Registry and where information was unclear the reporting clinician was contacted to obtain further information.
Results
Of 649 cases, congenital anomalies occurred in 173 (27%); 107 (62%) cases had one anomaly and 66 (38%) had two or more anomalies. Commonest anomalies included renal 35 (20%), heart 32 (18%), skeletal 32 (18%), short stature 30 (17%), small for gestational age (SGA) 28 (16%) and CNS 27 (15%). Of the 46XY, 46XX and 45X/46XY cases, anomalies were encountered in 113 (25%), 31 (26%), 19 (45%), respectively. In complete androgen insensitivity syndrome (AIS), congenital anomalies were reported in eight cases reported to have a mutation in the androgen receptor (AR) gene (ARmut + ve) (range of anomalies: renal, GI tract, heart, skeletal, skin) and in one case which was ARmut − ve (renal). Corresponding data for partial AIS: total 10 cases, 2 ARmut + ve, 3 ARmut − ve, 5 unknown. Of 89 cases of non-specific 46XY DSD, associated anomalies were encountered in 43 (48%). The range of anomalies included SGA 17 (40%), heart 10 (23%), CNS 8 (19%), renal 7 (16%), GI tract 6 (14%), ENT 5 (12%), skeletal 5 (12%), craniofacial 4 (9%), short stature 4 (9%), eyes 3 (7%), respiratory 3 (7%), skin 3 (7%), adrenal 1 (2%), haematological 1 (2%), and unidentified syndrome 1 (2%).
Conclusions
Associated congenital anomalies occur frequently in DSD, including in monogenic conditions such as AIS which are generally thought to solely affect sex development. These findings provide a direction for further study of genetic and environmental causes of DSD.

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Neoplasia, cancer and late effects

P149

Epigenetic modifiers reduce proliferation of human neuroendocrine tumour cell lines

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Neuroendocrine tumours (NETs), occurring at multiple sites including the pancreas, gastrointestinal tract, lung, thymus and pituitary, usually present at an advanced metastatic stage, and are increasing in incidence and prevalence as awareness and diagnostic techniques have improved. Treatments for NETs including surgery, drugs (e.g. somatostatin analogues), chemotherapy, radiotherapy and radionuclide therapy, are often not effective and as such additional therapeutic agents are required. We have assessed the efficacy of eight different compounds known to perturb functions of epigenetic related proteins, on the proliferation, over five days, of three NET cell lines (Bon-1 derived from a pancreatic NET, and H727 and H720 derived from lung NETs). We chose to study epigenetic modifiers as >40% of sporadic NETs are due to mutations of the multiple endocrine neoplasia type 1 (MEN1) gene, which encodes the histone methyltransferase MLL1 interacting protein, menin. Moreover, pancreatic NETs also have frequent mutations of chromatin remodelling genes, and pituitary NETs have alterations in histone modification. Two of the compounds, JQ1 and PFI-1, which are inhibitors of bromodomains found on bromo and extra terminal (BET) proteins, that selectively recognise ε-N-acetylated lysine residues, including those present on histone tails, and modulate the transcription of growth stimulating genes, were found to significantly reduce the proliferation of the three NET cell lines. JQ1 reduced proliferation by up to 95% ($P<0.0001$) and PFI-1 reduced proliferation by up to 40% ($P\leq0.0002$). The potency of these two compounds was subsequently assessed by a dose-titration study. To significantly reduce proliferation (i.e. to 50–60% of wild type) in the Bon-1, H727 and H720 NET cell lines, JQ1 and PFI-1 were required at low concentrations of 20–50 nM, and 100 nM–1 μM respectively. Thus, our data demonstrate that direct inhibitors of histone interacting enzymes are promising potential therapeutic targets for neuroendocrine tumours.

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P150

POMC correlates with viable tumour cell mass in lung cancer xenografts

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Small cell lung cancer (SCLC) is the most common cause of the ectopic ACTH syndrome and is characterised by rapid growth and poor prognosis. In the ectopic ACTH syndrome, tumours secrete highly elevated levels of the ACTH precursor, proopiomelanocortin (POMC), compared to ACTH itself (Oliver 2003). This study aimed to develop a mouse model of SCLC capable of secreting high levels of POMC and correlate this with viable tumour mass to assess POMC as a potential biomarker.

We have previously identified a panel of SCLC cell lines that express POMC. These tumour cells secrete POMC, but not ACTH, and express other neuroendocrine markers *in vitro*. When the human SCLC cell line, DMS 79, is grown *in vivo*, tumours stain positive for POMC and negative for ACTH. Tumours are also positive for additional neuroendocrine markers including neural cell adhesion molecule (N-CAM) and neuron-specific enolase (NSE). Tumour cells found invading into the muscle surrounding the tumour also stained positive for POMC.

Mice bearing DMS79 xenograft tumours exhibit high levels of POMC in the circulation. Over the 4 weeks tumours took to reach 1000 mm^3 in size, circulating POMC increased 11-fold. ACTH and corticosterone, although not correlating with tumour growth, both increased in relation to the time tumours were maintained *in vivo*.

Analysis of viable tumour mass in irradiated versus non-irradiated tumours revealed a strong correlation with POMC levels. Differences in viable cell number were a result of extensive necrosis in irradiated tumours.

These results indicate that POMC, but not ACTH, can act as a reliable indicator of viable tumour mass in patients with SCLC tumours capable of secreting POMC. The neuroendocrine phenotype may also play a role in SCLC tumour cell invasion.

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P151

Phenotype–genotype analysis in a cohort of patients with multiple endocrine neoplasia type 1 identifies a novel nonsense mutation at codon 554

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Aims
MEN1 is characterised by parathyroid, pituitary and pancreatic tumours in association with neoplasia of intra-thoracic endocrine tissue, adrenal glands and cutaneous manifestations. Mutations of the tumour suppressor Menin are causative and affected patients possess heterozygous germline mutations in MEN1, with acquisition of a second hit in the wild-type allele initiating tumourigenesis. Phenotype–genotype correlations can provide insights into the molecular function of Menin and help guide management and surveillance. We therefore sought to analyse patients with MEN1 presenting to a tertiary centre.
Methods
Case notes and electronic records were reviewed and those with a confirmed MEN1 phenotype were selected. Mutation loci and functional consequences were deduced using Ensembl and mutations were cross referenced with COSMIC and the Universal Mutation Database for MEN1.
Results
Of 48 patients, 41 patients had confirmed germline mutations in MEN1 identified by sequencing exons 2–10 (62%) or targeted screening (38%). One patient possessed wild-type MEN1 confirmed by multiplex ligation-dependent probe amplification. Results were pending or unavailable for six patients. Twenty-two different mutations were identified, with 10 in multiple family members. Mutations were present in exons 2, 3, 6, 7, 9 and 10 and introns 4, 6 and 9. Twenty-seven percent were missense, 27% nonsense and 36% frame-shift mutations. One mutation, Q554X in exon 10 was novel. The majority of patients developed PHPT and pituitary and pancreatic involvement were associated with mutations distributed throughout MEN1. Age of onset was variable.
Discussion
Our results reflect previous studies suggesting there are no obvious MEN1 phenotype-genotype correlations. A novel mutation, Q554X, was found in a patient with PHPT and a gastrinoma. The mutation is predicted to cause truncation at codon 554 resulting in loss of a nuclear localisation signal and C terminus which is thought to be critical for DNA binding and emphasises the importance of this region in Menin function.

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P152

Parathyroid gland studies in mouse models for endocrine tumours defines anatomical locations and ultrastructural differences between normal and tumour cells

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Investigation of parathyroids in mouse models is hampered by difficulties in identifying the small glands. We developed a microsurgical technique to identify murine parathyroids by dissecting from the distal thyrothymic ligament to the lower thyroid pole (LTP). Parathyroids were identified in 100 mice which comprised: 48 mice deleted for a cell-division-cycle 73 gene allele ($Cdc73^{+/-}$), involved in the hyperparathyroidism-jaw tumour syndrome; 10 mice deleted for a multiple endocrine neoplasia type 1 gene allele ($Men1^{+/-}$); and 42 wild-type controls ($Cdc73^{+/+}$; $Men1^{+/+}$). Parathyroid gland length and position relative to the LTP was assessed by histology. Parathyroid tumours from $Men1^{+/-}$ or $Cdc73^{+/-}$ mice were significantly larger than normal wild-type glands (mean length (±S.E.M.) was 762.8 μm (±62.0) in $Men1^{+/-}$ mice; 819.7 μm (±30.4) in $Cdc73^{+/-}$ mice; and 316.0 μm (±33.6) in $Cdc73^{+/+}$;$Men1^{+/+}$ mice, $P<0.0001$). The anatomical locations of the parathyroids relative to the LTP were as follows: ~40% were within 66.5 μm (±35.3) of the LTP; ~25% were 769.9 μm (±72.3) above the LTP in the posterior thyroid capsule; ~21% were 995.0 μm (±153.4) below the LTP in the thyrothymic ligament; ~7% were within the thyroid, 1275.3 μm (±125.4) above the LTP; and ~7% were within the thymus, 2233.3 μm (±68.8) below the LTP. Thus, ~35% of mouse parathyroids, as opposed to <10% of human parathyroids, are in the thyroid, thyrothymic ligament, or thymus. Electron microscopy revealed ultrastructural differences and demonstrated that $Men1^{+/-}$ parathyroid tumour cells compared to wild-type parathyroid cells: were smaller with a reduced cytoplasmic:nuclear ratio (2.57 (±0.30) vs 40.77 (±15.48), respectively, $P<0.05$); had more mitochondria per unit cytoplasmic area (13.35 (±1.12) vs 3.78 (±0.43), $P<0.0001$); and fewer secretory granules (4.75 (±0.67) vs 26.82 (±4.34), $P<0.0001$). This study, which represents the first anatomical location and ultrastructural characterisation of murine parathyroids, reveals that parathyroid tumours are more frequently located at ectopic sites, contain significantly more mitochondria, but have fewer secretory granules.

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Functional induction of the oestrogen-regulated PTTG1-binding factor in colorectal cancer

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PTTG1-binding factor (PBF) is an oestrogen-regulated proto-oncogene that is overexpressed in thyroid, breast and pituitary tumours. The precise role of PBF in tumourigenesis, however, has not been established, nor whether it is also an aetiological factor in non-endocrine cancer. In this study, we investigated PBF function in established colorectal cells and human tumours. Specific binding was evident between the tumour suppressor p53 and both endogenous and exogenous PBF in HCT116 cells. Half-life studies also showed that PBF overexpression significantly decreased p53 stability in HCT116 cells ($P<0.01$). In keeping with this, greater ubiquitination of p53 was detected in PBF-transfected HCT116 cells, with no effect on p53 transcription. To gain further insight we examined p53 expression and mutational status in 15 matched normal and cancer human colorectal specimens. Western blotting showed that the majority of colorectal tumours (14/15) had increased PBF, with a mean ~6-fold induction compared to normal tissue ($P<0.0001$). p53 mutations were identified in eight colorectal tumours, of which seven had highly stabilized p53. Tumours with mutated p53 were associated with high PBF expression, whereas those with wild-type p53 and high PBF expression had lower p53 ($P<0.05$). In addition, PBF expression was significantly higher ($P<0.05$) in colorectal tumours with extramural vascular invasion (EMVI), an independent predictor of recurrence and poorer overall survival. Despite being oestrogen-regulated, PBF expression was not significantly different between males and females ($P=0.15$). Our findings suggest that PBF's role in cell transformation most likely reflects its interaction with p53. This is the first study to demonstrate PBF overexpression in colorectal cancer, implying a role for PBF as a novel etiological marker in colorectal tumourigenesis, and revealing that PBF may also be involved in non-endocrine tumours.

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P154

PBF overexpression causes increased p53 ubiquitination and degradation via MDM2

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The pituitary tumor-transforming gene-binding factor (PBF) is a relatively uncharacterised proto-oncogene, which is overexpressed in thyroid tumours. PBF elicits tumor growth in nude mice, whilst thyroid targeted transgenic overexpression in the PBF-Tg mouse induces hyperplasia and macrofollicular lesions, accompanied by induction of the E2 ubiquitin ligase Rad6. Our previous unpublished data showed that PBF binds to p53, and reduces stimulation of downstream target genes by competitive binding. Further, half-life studies of p53 showed reduced p53 stability when PBF was overexpressed in K1 and TPC-1 thyroid papillary cancer cell lines, and ubiquitination assays confirmed this was due to increased ubiquitination and subsequent degradation by the proteasome. Now, GST pull-down assays demonstrate direct binding between PBF and MDM2, the principal negative regulator of p53. The competitive inhibitor of p53-MDM2 binding, Nutlin-3, revealed that the increased degradation of p53 observed when PBF was overexpressed was mediated by MDM2. No change in p53-MDM2 binding stringency was detected when PBF expression was ameliorated by siRNA treatment, and MDM2 subcellular localisation was unchanged by PBF overexpression in K1 cells. However, co-immunoprecipitation assays (TPC and K1 thyroid cells) revealed that PBF specifically interacts with Rad6, which has previously been shown to regulate p53 ubiquitination. Further, PBF-Tg mice demonstrated significantly induced genetic instability at 6 weeks of age, as determined by FISSR-PCR. Thus we propose that aberrantly expressed PBF functionally inactivates p53 via a complex interplay between Rad6 and MDM2, thus promoting genetic instability and tumorigenesis in thyroid cells and leading to thyroid cancer.

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P155

Mechanisms of estrogen receptor function in breast cancer

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Estrogen receptor (ER) is the defining feature of luminal breast cancers, where is functions as a transcription factor in response to the ligand estrogen. The traditional view of ER getting recruited to promoters of target genes is too simplistic. The recent discovery of ER–DNA interaction regions from ER+ breast cancer cell lines has revealed that ER rarely associates with promoter regions of target genes and instead associates with enhancer elements significant distances from the target genes. The genomic mapping of ER binding events also revealed the enrichment of DNA motifs for Forkhead factors. The Forkhead protein FOXA1 (HNF3a) was subsequently shown to bind to approximately half of the ER binding events in the genome and was required for ER to maintain interaction with DNA. We have extended on these findings to map ER binding events in primary breast cancers and distant metastases. We find context dependent ER *cis*-regulatory elements (cistromes) that give insight into underlying transcriptional networks. These differential ER binding profiles correlate with clinical response in ER+ breast cancers. We experimentally explore the binding dynamics between drug sensitive and resistant contexts and identify properties that govern ER binding differences. These data suggest that ER–DNA interactions are dynamic and can be modulated by changes in FOXA1. We are currently exploring mechanisms that mediate FOXA1–DNA interactions, in order to better understand ER transcriptional activity in breast cancer biology. This work provides insight into how estrogen mediates its effects in cancer and how hormone dependent cancers function after acquiring resistance to current endocrine therapies.

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P156

Investigation of the antiproliferative effect of natural sesquiterpene lactones on human cancer cell lines

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Plants and plant extracts play a crucial role in the research of novel antineoplastic agents. Five sesquiterpene lactones, 4β,15-dihydro-3-dehydrozaluzanin C (ddZC), zaluzanin C, artecanin, 3β-chloro-4α,10α-dihydroxy-1α,2α-epoxy-5α,7αH-guaia-11(13)-en-12,6α-olide and iso-secotanapartholide methyl ether were isolated from two traditionally used Asteraceae species (Onopordum acanthium and Artemisia asiatica). MTT-assay was used to determine the antiproliferative effect on human adherent cancer cell lines such as gynecological cell lines (HeLa, MCF7) and skin adenocarcinoma cell line (A431). The most effective compounds were further tested on HL-60 leukemia cell line by cell counting and reasonable IC_{50} values were obtained (3.6–13.5 μM). The two most effective natural products were subjected to additional tests in order to describe their apoptosis inducing capacity. Treatment with ddZC resulted in disturbance of cell cycle which was detected by means of flow cytometry. Concentration-dependent chromatin condensation and disruption of membrane integrity were detected after 24 h of incubation with 5 and 10 μM ddZC. Activation of caspase-3 by ddZC was additionally investigated. These experimental results indicate that sesquiterpene lactones and especially ddZC may be regarded as potential starting structures for development of novel anticancer agents.

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P157

Predicted NES in PBF appears to be functional *in vitro*

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Pituitary tumor transforming gene (PTTG) binding factor (PBF) is a proto-oncogene which is frequently upregulated in endocrine cancers. PBF has previously been determined to contain several putative signal sequences within its 180 amino acids. Previous studies have shown the nuclear localisation signal (NLS) to be functional and prediction software now suggests the presence of a putative leucine-rich nuclear export signal (NES) between residues 17 and 27. PBF is known to shuttle in and out of the nucleus, although as a small protein of ~25 kDa, this may be a passive process. Here, we hypothesised that the putative NES is functional *in vitro* and mediates active nuclear exit. Predicted NES residues were conserved in the PBF sequences of six out of seven analysed species. The most resounding NES prediction was present in human PBF, with an average NES score of 0.69 over the 11 consensus NES residues of sequence LXXXLXXLXL, where 'L' is a hydrophobic residue (often leucine) and 'X' is any other amino acid. Exportin-1 (CRM1), the central protein involved in nuclear export, was chemically inhibited and knocked down via siRNA in COS-7 and K1 cells, and the location of PBF assessed using immunofluorescence. When CRM1 was knocked down, cytoplasmic PBF levels were reduced as, more unexpectedly, were nucleolar levels. Fibrillarin, used as a marker of cellular nucleoli, co-localised with PBF in the presence of scrambled siRNA. However, when CRM1 was specifically knocked down, this co-localisation was no longer present, and there was a marked reduction in PBF staining corresponding with nucleolar location. Consistent findings were apparent with the CRM1 inhibitor, Leptomycin B. Thus, although the precise nuclear, nucleolar and cytoplasmic functions of PBF remain to be delineated, these data suggest that PBF has a conserved and functional nuclear export sequence, revealing that PBF exit from the nucleus is governed by an active process.

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P158

Pituitary metasases: patients presenting with cranial nerve palsies and diabetes insipidus: a single centre experience

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Background
Pituitary metastases are a rare complication of systemic malignancy. The most common presentations of pituitary masses include visual field defects, headaches, and hypopituitarism, but cranial nerve palsies and diabetes insipidus are also recognised although unusual. We aimed to determine if these were more frequently associated with pituitary metastasis.
Methods
We conducted a review of 944 patients undergoing pituitary surgery from a teaching hospital neuropathology database 1997–2012, identifying histologically confirmed pituitary metastasis or deposit from haematological malignancy. We reviewed symptoms at presentation, including diabetes insipidus, cranial nerve palsies and anterior pituitary hormonal deficiencies. In addition, we recorded the site of primary tumour.
Results
We identified 11 cases of metastatic pituitary lesions, representing 1.16% of all surgical biopsies performed. Breast and lung cancer were the most common primary neoplasms metastasising to the pituitary (36.3 and 27.3% respectively). The remaining neoplasms metastasising to the pituitary were liver and kidney cancer, and haematological malignancies including myeloma and B-cell lymphoma. The most common presentation was headache and visual field defects. However, 63.6% of patients presented with anterior pituitary deficiencies, 54.4% with cranial nerve palsies, and 27.2% with diabetes insipidus. Primary pituitary presentations occurred in 45.4% of cases prior to a diagnosis of malignancy. In cases where there was a known history of malignancy, the mean time between diagnosis of primary tumour and the onset of pituitary symptoms was 26 months (SE = 12.3, $n = 6$) with a median of 15 months.
Conclusion
Cranial nerve palsies and diabetes insipidus are highly unusual in patients with pituitary adenoma and should alert clinicians to consider and investigate for pituitary metastasis in patients with or without a previous diagnosis of neoplastic disease.

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P159

The relationship between anatomical location of phaeochromocytoma and paraganglioma and their secretory properties

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Background
Phaeochromocytomas (PCC) and paragangliomas (PGL) are neuroendocrine tumours that can secrete catecholamines. PCC are found in the adrenals, whereas PGL are found at any level between the skull base to the pelvis. More than 25% of cases are associated with mutations in susceptibility genes such as the SDH subunits. Screening for PCC/PGL crucially depends on detection of biochemical markers such as catecholamines and metanephrines (catecholamine metabolites).
Study design
This retrospective study looked at the influence of the site of PGL/PCC on their secretory properties. The relationship between the type of genetic mutations and the secretory properties of PGL/PCC was also investigated. 103 patients with PGL/PCC were identified. Secretory tumours were those with pre-operative levels of catecholamine or metanephrines above normal reference ranges.
Results
PCC are almost exclusively secretory (97%, $P < 0.0001$) while PGL can be secretory (52%) or non-secretory (48%). The majority of the secretory tumours were located in the thorax/abdomen (97%, $P < 0.0001$). In contrast, most H&N PGL are non-secretory (80%). Interestingly, both patients with secretory H&N tumours in this study carried germline mutations in SDH-B, which may lead to metachronous tumours or undetected metastases. In addition, as shown in this

study, PGL/PCC that occur in patients with SDH-B mutation tend to be secretory (77%), whereas the tumours could be either secretory (50%) or non-secretory (50%) in patients with SDH-D mutations. Although no additional tumours have been detected in these two patients on follow-up, further and close monitoring is necessary.

Conclusions

PCC are usually secretory whereas PGL can be either secretory or non-secretory. Secretory PGL/PCC tend to be sited in the abdomen. H&N PGL are generally non-secreting tumours. Most importantly, normal catecholamine or metanephrine results do not exclude the diagnosis of PGL/PCC.

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P160

Adjuvant use of ^{131}I-MIBG in phaeochromocytoma and paraganglioma at high risk of malignancy

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Phaeochromocytomas (Phaeo) and paragangliomas (PGL) are rare catecholamine producing tumours. It can be difficult to predict their malignant potential and patients can sometimes present with metastatic disease many years after their original diagnosis (1). Therefore, we offer a single dose of adjuvant ^{131}I-Metaiodobenzylguanidine (^{131}I-MIBG) to subjects felt to be at higher risk of local recurrence (demonstration of capsular or vascular invasion on pathology). However, there is a lack of evidence supporting this practice and concern regarding the safety and tolerability of such therapy. We therefore aimed to review outcomes of cases of adjuvant ^{131}I-MIBG use in such 'high risk' patients. We reviewed the casenotes of patients who received adjuvant ^{131}I-MIBG for phaeo or PGL from 1985 to 2010. Patients who had metastatic disease at the time of treatment were excluded. Recurrence of disease was assessed by ^{131}I-MIBG scintigraphy, computed tomography/magnetic resonance imaging, biochemical measurement of catecholamines and symptom recurrence.

Fifteen patients (eight males) were reviewed. Mean age was 36 years (range 9–79). One patient was receiving an α blocker and another was on β and α blockers during treatment. All had undergone surgical resection prior to ^{131}I-MIBG therapy. Indications for therapy were capsule breach, local nodal spread/vascular invasion or local recurrence. One patient received ^{131}I-MIBG due to a strong family history of malignant disease. Each patient received one dose of ^{131}I-MIBG; mean dose was 9166 MBq (range 5180–10 353 MBq). The treatment was well tolerated with no haematological sequelae. One patient developed recurrent disease and one patient died due to disease progression. Follow-up is ongoing for the rest of the cohort with no evidence of recurrence.

Adjuvant ^{131}I-MIBG treatment of phaeo/PGL is well tolerated and routine use of α/β blockade during therapy is not required. These data support its use in an adjuvant basis to prevent progression to metastatic disease in patients thought to be at risk of malignant disease however lack of a control group prevents firm conclusions.

Reference

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P161

Multi-modal approach to treatment in advanced adrenocortical carcinoma

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A 38-year-old lady, who was 9 months *post-partum*, presented in 2008 with hirsutism, acne and abdominal discomfort. She was virilised and had an easily palpable right upper quadrant mass. Biochemistry revealed mild hypokalaemia (K 3.3 mmol/l), low albumin and gross elevation of serum androgens (androstenedione 93.9 nmol/l, DHAS 37.7 μmol/l and testosterone 13.7 nmol/l). UFC was 380 nmol/24 h but following 1 mg dexamethasone, cortisol suppressed fully. Urine steroid profile demonstrated increased excretion of androgen and progestogen metabolites and grossly elevated THS excretion. Renin and aldosterone were normal. On CT scanning a large right adrenal tumour was seen with liver metastases encompassing the IVC. Initial treatment comprised mitotane and operative de-bulking. Histology confirmed adrenocortical carcinoma (ACC), so she underwent four cycles of chemotherapy with mitotane + doxorubicin/ cisplatin/ etoposide, followed by radical radiotherapy to the adrenal bed (50 Gy in 25 fractions over 5 weeks). She was maintained on mitotane but in 2010 relapsed with liver metastases which were not amenable to further surgery. Radiofrequency ablation (RFA) of the liver metastases was then performed. In 2011 she developed lung and progressive liver metastases. Further RFA to liver and lung was administered, followed by repeated chemotherapy (with the same regimen), but disease progressed further. Sunitinib was initiated and the dose increased, which resulted in a partial response, although an area of apparent selective resistance in part of the liver was treated by nanoknife ablation. Recent CT suggests some disease progression, although androgens have normalised over the years (testosterone <0.5 nmol/l), a'dione 4.7 nmol/l, DHAS 2.9 μmol/l). There are little published data on RFA in metastatic ACC, but two small studies (both $n=8$) have demonstrated safety and short-term efficacy in terms of tumour shrinkage (Wood *et al*.), and median survival of 1.9 years post RFA (Ripely *et al*.) respectively. Pre-clinical studies exploring the use of sunitinib in ACC are promising (Lin *et al*., Kroiss *et al*.) and there are a few published cases and a small clinical trial of use of sunitinib in metastatic ACC reporting variable outcomes (Lee *et al*., Kroiss *et al*.). This is a highly unusual case of prolonged survival in aggressive ACC with the use of multiple therapeutic modalities, many of which have a paucity of clinical evidence.

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P162

Prevalence of multiple endocrine neoplasia type 1 syndrome in primary hyperparathyroidism

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Objective

To assess the prevalence of multiple endocrine neoplasia type 1 (MEN1) in patients with symptomatic primary hyperparathyroidism (PHPT).

Methods

A retrospective analysis of 75 consecutive patients with symptomatic PHPT from January 1994 to July 2012 was done, who underwent parathyroid surgery at our centre. Five patients had MEN1 syndrome. Among them one was familial MEN1. The patients with MEN1 were analyzed based on clinical presentation, biochemical and hormonal profile, imaging modalities and treatment outcome.

Results

Mean age of the study patients was 28.6 ± 12.9 years (male:female = 4:1). Mean age of the rest all patients was $43.5 + 11.5$ years. Four were symptomatic at presentation and one was diagnosed on family screening. Mean duration of symptoms was 23.8 ± 12.1 months. Bone pains and painful proximal myopathy were the commonest presentation (4/4), followed by pathological fractures in one case. Distal renal tubular acidosis was diagnosed in one case, which normalized after surgery. The most common presenting manifestation was PHPT in four patients (80%), followed by hyperprolactinemia due to pituitary tumor in one patient (20%). PHPT was a universal feature (100%) in all MEN1 syndrome followed by pituitary tumors in three cases (60%) and enteropancreatic neuroendocrine tumors in two cases (40%), with both being insulinoma. Among the pituitary tumors, prolactinoma and nonfunctioning pituitary adenoma were present in two each cases demonstrating equal prevalence.

All PHPT patients underwent parathyroidectomy and the ones with MEN1 had mean parathyroid gland weight was 1235.6 ± 684.5 mg, which was larger than the rest (Mean parathyroid gland weight was 835.4 ± 178.5 mg, $P=0.04$). Three PHPT patients with MEN1 syndrome had double adenoma and two patients had multiglandular parathyroid involvement.

Discussion

PHPT Patients with MEN1 tend to be younger with multifocal involvement and larger glands. Eighty % of MEN cases had PHPT as initial manifestation followed by hyperprolactinemia in 20% cases. Our series demonstrated higher incidence of symptomatic PHPT, higher prevalence of pituitary tumors (80%) and insulinoma (40%). Additionally prevalence of pituitary involvement (80%) outscores the prevalence of enteropancreatic neuroendocrine tumors (40%). These deviations from classic involvement depicted in literature could be due to small sample size of the study population.

Conclusion

All young patients with double adenoma or multiglandular parathyroid

involvement should be screened for MEN1 syndrome irrespective of the symptoms. To avoid the recurrent surgical procedure, high index of suspicion is needed for diagnosis.

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P163

Primary thyroid cancer as late effects of childhood cancer therapy: a case series of five patients

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Introduction
Advancement in modalities of treatment in childhood malignancies has improved the survival. Though Hypothyroidism is the first recognised and commonest thyroid disease in these set of patients, there is also an increased risk of subsequent primary thyroid cancer among the Survivors childhood malignancies who have had radiotherapy to the head, neck, or upper thorax. We hereby present case series of five patients who developed thyroid cancer after childhood cancer therapy.
The average age group of the patients is 27.4 (24–39). Three of these had ALL, two other patients had Hodgkin's disease and AML each. Average age at diagnosis of primary malignancy was 11 (1–25 years) and average duration between treatment of primary malignancy and diagnosis of thyroid cancer was 13.2 (7–21 years). Of the three patients with ALL, one had cranial irradiation with initial chemotherapy and two others had Total body radiation. Patient with AML had total body radiation before allogeneic bone marrow transplant and one with Hodgkin's disease had chemotherapy only. Three of these patients presented with thyroid lump and two others were diagnosed on ultrasound scan. All five patients had papillary thyroid cancer. Only one of these had local metastasis to lymph nodes. All patients had total thyroidectomy with level six neck dissection followed by Radio iodine ablation.
Conclusion
Papillary thyroid cancer is commonest type of thyroid cancer in survivors from childhood cancer therapy. Radiation increases the risk for development of thyroid cancer. Standard long-term follow-up of patients treated for childhood malignancies particularly requiring radiation to head and neck region should include thyroid ultrasound for early detection of the cancer.

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P164

Diagnosis, localisation and management of insulinoma; a single-centre experience

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Nineteen cases of insulinoma were treated in this centre between 2003 and 2012 (12 female, 7 male, 5 associated with MEN-1). Of the cohort, 14 had their primary investigation and management here. All presented with symptoms consistent with hypoglycaemia and had a supervised fast demonstrating serum glucose <2.2 mmol/l with inappropriately normal/elevated insulin.
11 of 14 patients proceeded to surgery. Two elderly patients declined further investigation after their fast and CT scan, which identified a lesion in both cases. Conservative management with somatostatin analogues/dietary interventions were successful in managing hypoglycaemia. A third case had radiological evidence of extensive liver metastases and underwent hepatic arterial embolisation along with palliative chemotherapy but died 12 months after diagnosis.
Of the 11 patients who proceeded to surgery, the lesion was localised pre-operatively in each case, all lesions being confirmed as insulinoma on histology. All of these patients were cured of their symptomatic hypoglycaemia with no recurrence to date (follow-up range 3 months–9 years). Ten patients had CT as first line imaging, with the lesion identified in eight cases (sensitivity 80%). These lesions were subsequently seen on EUS. Eleven patients underwent EUS and all 11 procedures identified a lesion (100% sensitivity). FNA was carried out in addition to EUS in two cases, cytology demonstrating grade 1 neuroendocrine tumour. Five patients had MRI, with successful localisation in all cases. Coeliac axis angiography yielded a sensitivity of 57% over seven cases. Where this failed to demonstrate a lesion, selective calcium arterial stimulation and venous sampling was successful with 100% sensitivity (four cases, one of which was concordant with the angiography result). Specificity was 100% for all modalities. Our experience suggests, in our centre, a combination of MRI/CT imaging and EUS following a biochemical diagnosis of insulinoma accurately identifies lesions, facilitating targeted curative surgery.

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P165

Audit of patients with multiple endocrine neoplasia type 1 in a tertiary referral centre

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Aim
To review the presentation, management and outcomes in adult patients with MEN1 attending a multidisciplinary clinic.
Methods
Case notes and electronic records were reviewed in patients attending a tertiary centre clinic for care of MEN1.
Results
Forty-eight patients were analysed; 46% were male and 54% female. Mean age was 49 years (range 14–89) and 4% were deceased. Eighty-five percent had confirmed MEN1 mutations and 23% appeared to have sporadic mutations.
Eighty eight percent had developed PHPT (mean age 42 years, range 14–82). Thirty three percent had developed renal stones and 17% osteoporosis. Seventy-nine percent were treated surgically and were histologically determined to have hyperplasia (27%), adenomas (23%), mixed hyperplasia-adenoma (14%) and otherwise unknown (26%). Cinacalcet was used in 4% patients for resistant disease post-surgery. Thirty-two patients (67%) had pancreatic NETs (mean age 43 years, range 16–72). Of the 16 patients with gastrinomas, eight had metastatic disease. Six gastrinomas were surgically resected (including four metastatic tumours). One patient died as a result of a metastatic gastrinoma. Four patients had insulinomas (all of which were treated surgically), and two had glucagonomas. Thirteen patients (27%) developed pituitary adenomas (mean age 48 years, range 23–60). Of these, six were prolactinomas (five macro, one micro), two were corticotroph adenomas and five were non-functioning. Hypopituitarism was found in five cases (two hypothyroidism, three hypogonadism). Three patients underwent curative transsphenoidal surgery; five patients were successfully managed with dopamine agonist therapy alone.
Additionally, two patients had thymic masses, one patient had multiple gastric carcinoid tumours, and there was one case of DIPNEC.
Discussion
The characteristics of MEN1 patients presenting to our service is consistent with previous reports (Pieterman *et al.* 2011), except presentation with PHPT was older; this may explain the higher frequency of renal stones observed. Optimal management of gastrinomas in MEN-1 remains unclear. Multicentre studies are needed to help guide treatment.

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P166

Difficulties in management of malignant insulinoma

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Introduction
Insulinomas are the most common, functioning, pancreatic neuro-endocrine tumours. The minority of patients $<10\%$ who present with metastatic disease have a median survival of <2 years.
We present a case of a gentleman with a 30 years history of Multiple Endocrine

neoplasia type 1 (MEN1), which highlights the various modalities of treatment and the challenges from his progressive disease and marked symptomatic hypoglycaemia.
Case History
Mr R W, a 75-year-old patient was first diagnosed with primary hyperparathyroidism in 1972. He underwent parathyroidectomy and had a recurrence in 2002 requiring repeat surgery. Genetic testing confirmed MEN-1. His son also was found to have MEN-1. In 2005, he was diagnosed with Insulinoma with symptomatic hypoglycaemic episodes. At the time of diagnosis, he had metastatic lesions in the liver and spleen in addition to the pancreatic lesion.
In 2006 he underwent surgery including distal pancreatectomy, splenectomy and resection of liver lesions. In addition radiofrequency ablation of liver lesions was also performed. Due to his clinical and radiologically progressive disease, he underwent three further radiofrequency ablations in 2007. An Octreotide trial in 2008 failed to give symptomatic relief. Progressive disease necessitated another surgery in 2009, followed by further radiofrequency ablation.
Diazoxide provided limited symptomatic relief but with significant side effects.
In view of his on-going hypoglycaemic episodes and inability to tolerate higher doses of diazoxide, he was initiated on Everolimus in June 2012. We show, with continuous glucose monitoring, that the frequency of his hypoglycaemic episodes has reduced significantly. It is too early to comment on disease resolution.
Conclusions
Everolimus is a highly promising treatment for metastatic insulinoma that combines direct anti-tumour effects with hyperglycaemic effects that serve to relieve the morbidity from hypoglycaemia.

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P167

Crizotinib induced hypogonadism: a novel complication of lung cancer treatment

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Tyrosine kinase inhibitors and other targeted treatments are revolutionizing the treatment of cancer. However multiple endocrine side effects of these treatments are emerging.
Crizotinib, a multi-targeted small molecule tyrosine kinase inhibitor of ALK and c-met, has been approved by the FDA for the treatment of non small cell lung cancer (NSCLC) patients with a novel oncogenic gene fusion, EML4-ALK and its variants. Crizotinib is generally well tolerated. Interestingly, hypogonadism has been observed in patients treated with crizotinib. Weickhardt *et al*. reduced testosterone level in all NSCLC patients treated with crizotinib compared to only 32% of those not receiving the drug. This occurred as early as 2–3 weeks after treatment initiation with rapid improvement after treatment interruption. The mechanism of crizotinib-induced hypogonadism is unclear with features of both primary and secondary hypogonadism, with some, but not all patients having low gonadotrophins.
We present the case of a 35 years old gentleman with metastatic NSCLC receiving treatment with crizotinib. He described progressive lethargy and loss of libido. Investigations revealed a low testosterone of 5.6 nmol/l and very low SHBG of 8 nmol/l. LH was normal at 4.6 IU/l but FSH was high at 12 IU/l. Prolactin, whilst taking metoclopramide was also elevated but an MRI of his pituitary did not show any evidence of metastases or other pituitary mass.
He therefore appears to have isolated hypogonadism most likely secondary to crizotinib therapy, although the low SHBG has not previously been described with this drug. He has now commenced a trial of testosterone replacement.
This illustrates the importance of endocrinologists being aware of the side effects of novel anti-cancer drugs and of joint working between oncologists and endocrinologists.

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P168

Review of patients with adrenocortical carcinoma at a tertiary referral centre

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Introduction
Adrenocortical carcinoma (ACC) is a rare but devastating malignancy. We performed a review to determine outcome in our patient cohort.
Of 20 patients 14 were females, six males. Median overall survival was 27.5 months (range 1–168), nine being deceased. Mean age at presentation 52.3 years (range 18–71). The majority of cancers were large; 5% $\leq$5 cm, 40% 5–10 cm, 55 $>$10 cm. 25% had stage IV disease, 10% stage III, 55% stage II, 10% stage 1. 75% of 16 with histology had Weiss score $>$2. The functionality of tumours was determined using blood tests and urinary steroid profiles. 70% were functioning. 67% tumours in males were secretory (glucocortiocoids only). 93% tumours in females secreted a range of hormones; glucocorticoids 41%, androgens 41%, mineralocorticoids 11%, other precursors 11%.
Of 16 adrenalectomies, five underwent laparoscopic adrenalectomy, eight laparotomy, three procedures unknown. Surgical procedure did not affect survival.
Fifteen patients were treated with mitotane; seven as adjuvant therapy after surgery, six combined with chemotherapy, two as monotherapy for recurrence when unfit for chemotherapy. Mean duration of Mitotane was 18.3 months (range 1–70). Therapeutic level (14–20 μg/dl) was achieved in only five due to side effects.
One patient underwent post-operative radiotherapy to the adrenal bed. Seven patients had palliative chemotherapy with EDP (Etoposide, Doxorubicin, Cisplatin) and Mitone 1st line. Two required 2nd line Streptozocin regimes. Median overall survival was 18 months (range 2–30). Eleven of all patients suffered adverse effects to systemic therapies.
Of 11 patients under surveillance 6 are in remission, 3 having completed $>$2 years mitotane at therapeutic levels. Five patients are undergoing treatment for recurrence or disease progression, three undergoing palliative chemotherapy.
Conclusions
In our small cohort, ACC has a poor prognosis. Further multicentre studies are needed to answer outstanding questions regarding adjuvant mitotane therapy. New treatments are needed to improve the dismal prognosis.

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P169

Between genetics, guidelines and treatment in MEN2A: a family affair

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The multiple endocrine neoplasia syndrome type 2A (MEN 2A) is the consequence of a heritable mutation in the RET proto-oncogene, leading to a very high predisposition to develop medullary thyroid carcinoma (MTC), pheochromocytoma and primary parathyroid hyperplasia. Screening of 'at-risk' family members and prophylactic thyroidectomy is recommended – but not always easily accomplished.
Our proband was diagnosed with MTC in 1977 (at age 21), had a subtotal thyroidectomy and was lost to follow up until 1998 when he was discovered to have a calcitonin of $>$1000 pg/ml, but no therapy was pursued. In 2000, while admitted for acute pancreatitis, a 3 cm left adrenal mass was discovered on CT; the patient reported being hypertensive since age 18 (TAs = 160 mmHg), without the spells suggestive of pheochromocytoma, but had high urinary metanephrines (1200 μg/24 h). MEN2A was first considered after he underwent left adrenalectomy, and pathology confirmed pheochromocytoma. By then age 46, he brought his two sons to the endocrine clinic – aged 22 and 14 years. Genetic testing revealed a mutation in codon 634 of the RET proto-oncogene. Both boys had marginally elevated calcitonin levels, therefore thyroidectomy was recommended, but both refused and signed. The grandfather, 75 years old and known to be diabetic, hypertensive and with coronary artery disease, carried the RET mutation and was diagnosed with pheochromocytoma (bilateral adrenal masses of 40/17 and 42/26 mm) and MTC (calcitonin 1200 pg/ml), as an example of the natural history of the disease. However, all cases were reluctant to pursue surgery until pheochromocytoma triggered admission into the hospital. Current treatment (unfortunately delayed too long) according to guidelines in this family is now in progress.
Our case series suggests that although the biology of MEN2A is currently better understood, with clear clinical guidelines certified by costly investigations, the recommended therapy is not always easily followed, with non-compliance being probably multifactorial.

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Cowden syndrome

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A 27-year-old lady presented with headache, bilateral papilloedema and long standing thyroid enlargement. Her MRI brain showed a cerebellar lesion. She had surgery and histology was consistent with Lhermitte–Duclos, a benign brain tumour. She also had retinal changes. Her fluroscein angiogram and optical coherence tomography showed multiple retinal harmatomas and pigmented retinal epithelium. She had retrosternal thyroid extension and had total thyroidectomy because of tracheal compression. In view of brain histology and goitre, Cowden syndrome was suspected. Genetic analysis confirmed PTEN gene mutation and clinical diagnosis.

Cowden syndrome is a rare autosomal dominant condition characterised by multiple harmatomas affecting multiple organs including endocrine glands, skin and mucous membrane. The condition develops due to a mutation in tumour suppressor gene PTEN on chromosome 10q23. PTEN is responsible for cell growth, apoptosis and cell migration. It has near complete penetrance which becomes evident by age 20 in most affected individuals. There is increased incidence of certain type of malignancies, the commonest being breast (25–50%), non medullary thyroid cancer (10%) and endometrium carcinomas (5–10%).

Surveillance is vital in patients with confirmed PTEN mutation. The difficulty arises as there are variable phenotypes and age-related penetrance. Based on NCCN recommendation (2009), a protocol for screening and surveillance of Cowden syndrome has now been developed by St George's Genetic Department.

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P171

Recurrent bronchial carcinoid tumour presenting as a thyroid nodule

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A 38-year-old lady presented in 2003 with a 2 years history of recurrent pneumonia, with CXR having demonstrated intermittent right lower zone consolidation. CT of chest revealed dense right lower lobe consolidation and a 2.5 cm tumour occluding the right lower lobe orifice was found on bronchoscopy. Strongly positive immunohistochemical staining for chromogranin, NCAM, PGP 9.5 and synaptophysin suggested carcinoid so right lower and middle lobectomy was performed. Histology confirmed a classical carcinoid tumour with complete resection margins. She was discharged from follow up and remained well until 2011 when she was referred to our clinic with a right sided neck swelling associated with weight loss and back pain. The thyroid was enlarged with a firm nodular lump on the right side, and palpable cervical lymph nodes. BP was normal and she was clinically and biochemically euthyroid. Ultrasound revealed multiple suspicious lesions within the thyroid which appeared highly vascular. Enlarged lymph nodes were seen within the neck. Core biopsy suggested recurrent neuroendocrine tumour (NET), and subsequent imaging showed metastatic disease in the mediastinum, abdomen and skeleton. Biochemistry revealed elevated chromogranin A >300 pmol/l, calcitonin <14 ng/l, and normal urinary 5HIAA, catecholamines and metanephrines. Further imaging comprised octreotide scan which showed normal uptake, MIBG scan which found both lobes of the thyroid and some of liver lesions to be MIBG avid, although most of the metastases were not, raising the possibility of two synchronous cancers. FDG-PET scan, however, showed equally FDG avid disease in the skeleton, liver, subcarinal nodes and thyroid. The patient's main symptom is back pain, so she was treated with radiotherapy to T11 (20 Gy in five fractions) and is receiving opiates and monthly pamidronate. She is eligible for a clinical trial examining the efficacy of everolimus vs. placebo. Octreotide has not been used therapeutically because she has neither symptoms of carcinoid syndrome nor octreotide avid disease on scanning. Carcinoid tumour presenting or recurring as a thyroid nodule is extremely rare, with very few cases previously reported. Although bronchial carcinoid tumours are typically indolent, this case highlights the importance of long term follow up of all patients with these tumours, an approach which is now recommended in a number of consensus guidelines.

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P172

Gut carcinoid in a patient with horseshoe kidney and family history of carcinoid syndrome: a case report

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Introduction

The familial risks of carcinoids are not clear. There has never been a report of gastrointestinal carcinoid coexisting with horseshoe kidney.

Case presentation

We present the case of a 15 mm well differentiated metastatic small bowel NET with vascular and perineural invasion and three of four positive lymph nodes (Ki67: 1.9%, ENETS stage: pT4 pN1 pMX R1) in a 75-year-old British male with episodes of diarrhea and 24 h urinary 5HIAA: 48 (<40), pancreatic polypeptide: 955 ng/l (0–200), CgA: 50 U/l (0–30), N-terminal glucagon: 295 ng/l (0–250), C-terminal glucagon: 165 ng/l (0–150); gastrin, neurokinin A, VIP, insulin, somatostatin and serum calcium were normal and features of MEN1 absent. Interestingly, the patient had horseshoe kidney and family history of a sister with lung carcinoid (age 53), and 11 years later metastatic small bowel carcinoid and carcinoid syndrome (Ki67: 1%, CgA and urinary 5HIAA slightly elevated). The review of the literature shows that the development of primary carcinoid tumor within horseshoe kidney might be 60–85-fold higher compared to normal kidneys, while coexistence of gastrointestinal carcinoid with horseshoe kidney has never been reported so far. Previous studies using the Swedish Family-Cancer Database estimated standardized incidence ratios for offspring when their parents had a carcinoid of 4.35 ($n=8$, 95% CI 1.86–7.89) for small intestinal and of 4.65 ($n=4$, 95% CI 1.21–10.32) for colon carcinoids.

Conclusions

The case presented is the first to report coexistence of gastrointestinal carcinoid with horseshoe kidney. The familial aggregation of gastrointestinal carcinoid might underlie the need of screening programmes and gene finding studies.

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Insulinoma in postprandial hypoglycaemia and aggressive behaviour

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Background

Fasting hypoglycaemia is a common presenting symptom of insulinoma. However, insulinoma should be considered as a potential cause in those presenting with symptoms of hypoglycaemia after meal (1, 2, 3). Here we report a case who initially presented with postprandial symptoms though there was evidence of fasting hypoglycaemia subsequently.

Case report

A 57-year-old lady initially presented with a 2 years history of palpitation, feeling hot, sweating and dizzy episodes which occurred within 2 h of meals. She also gained some weight. There was no history of nocturnal or fasting hypoglycaemia. Past medical history included CREST syndrome. There was no family history of note. On examination she has a BMI of 34.5.

Thyroid function tests, plasma metanephrines, short synacthen test, urea and electrolytes, renal and liver function tests were normal. Further monitoring of capillary glucose revealed fasting hypoglycaemia.

Subsequently, a supervised 48-h fast test was performed. Patient developed hypoglycaemia within first 12 h. The lowest blood glucose level was 2.2 mmol/l. There were elevated insulin level 49 pmol/l and pro-insulin level 22.2 pmol/l. C-peptide level was 863 pmol/l. Sulphonylurea screen was negative. Hypoglycemia was associated with neuroglycopenic symptoms particularly very aggressive behaviour.

CT scan of the abdomen and endoscopic ultrasound (EUS) have confirmed 1.2 mm solitary insulinoma in the tail of pancreas.

Conclusion

This case has highlighted the importance of awareness of post prandial hypogycemic symptoms as presenting feature of insulinoma.

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P174

Hyperparathyroid jaw tumour syndrome
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Hyperparathyroid Jaw tumour syndrome is a familial form of primary hyperparathyroidism. Individuals are predisposed to develop parathyroid carcinomas (15%), ossifying fibromas of mandible and maxilla (30%), renal abnormalities including cystic lesions and hamartomas, and uterine tumours (1, 2). The pathogenic mutation is in CDC73 gene (previously known as HRPT2 and C1orf28) inherited in an autosomal dominant manner.
Our patient was the first person in the UK to have the diagnosis confirmed on genetic testing. Two sisters had primary hyperparathyroidism. Father had hyperparathyroidism and an ossifying fibroma. During subsequent follow up, she was found to have a small neuroendocrine tumour of her pancreas and is under regular review. The proposed screening protocol is discussed.
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Nursing practice

P175

Using a nursing model in the management of a patient with McCune–Albright syndrome
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McCune–Albright syndrome and fibrous dysplasia are a rare sporadic skeletal disorder in which normal bone structures and marrow are replaced by a benign fibrous tissue. The disease can be limited to one or many bones and is more prevalent in females. One of the clinical consequences experienced by patients is severe bone pain for which bisphosphonates are an effective treatment.
By the use of a nursing model for the treatment of the patient with this rare disease it can enable the holistic care of the patient. Roper, Logan and Tierney (1980) is one of the many nursing models it uses 12 components and is based on the activities of daily living, which can be utilised to ensure all the components of a patients care can be encompassed. The patient assessed in the outpatient setting is a female who has a severe form of the disease she has scoliosis, is wheelchair bound, suffered many fractures, had a hysterectomy and breast cancer. When reviewing the patient it is important that the holistic care of the patient is taken into account, not just the treatment of the medical condition.
The nurses role is pivotal to the positive experience of the patient, and builds a meaningful relationship in the management of this rare disorder. As a specialist nurse it is essential that the patient has confidence in the ability of the nurse to provide expert advice and effective communication so that the patient feels comfortable contacting the helpline when she is suffering with pain and that the nurse has systems in place and so can expedite admission to the Programmed investigation unit, taking into account the complexities of the disease and the practicalities of attending the hospital such as transportation, dietary needs, and eliminating, and building a rapport with the patient and showing compassion.
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Development of adult endocrine specialist nurse competencies
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The Society for Endocrinology Nurse Committee provides national, international and local guidance, support and networking for nurses working in Endocrinology. Following review and revalidation of the endocrine nurse certificate, the Nurse Committee looked to explicate adult endocrine nurse competencies. The need for core competencies to standardise role expectations was a concern voiced from nurses and committee members. Up to the introduction of paediatric endocrine nurse competencies (RCN 2008) there had been no formal framework for endocrine nurses. Many specialist nurses work in isolation and the development of competencies could; assist those new to post, facilitate continuing professional development, assist managers with identifying strengths and gaps in knowledge, skills and services, aid performance appraisal and thereby improve and support endocrine nurses locally by providing a path of career progression. A working group was formed consisting of eight experienced endocrine nurses working in the NHS and private sector. Eight improve patient care. The competencies are to be launched in 2013 when they will be introduced and made available to all endocrine nurses and endocrine centres in the UK. The document will also be accessible via the Society for Endocrinology website reaching a wider population and international audience.
Declaration of funding
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Obesity, diabetes, metabolism and cardiovascular

P177

Association of calcium-sensing receptor polymorphisms with vascular calcification and glucose homeostasis in renal transplant recipients
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The calcium-sensing receptor (CaSR) is a G-protein coupled receptor that regulates extracellular calcium concentration. The CaSR is also implicated in the pathogenesis of non-calcium disorders such as vascular calcification and diabetes, which are leading causes of cardiovascular disease. Common CaSR single nucleotide polymorphisms (SNPs) have been demonstrated to be determinants of calcium metabolism. The aim of this study was to investigate whether CaSR SNPs may influence vascular calcification in renal transplant recipients, a patient group in which cardiovascular disease is the major cause of death. Three coding region polymorphisms (Ala986Ser, Arg990Gly, Gln1011Glu) and three CaSR promoter polymorphism (rs115759455, rs7652589, rs150189) were analyzed in 285 renal transplant recipients that had undergone radiological assessment of aortic medial and coronary artery intimal calcification, vessel wall stiffness, and measurement of calcification risk factors including blood pressure, renal function, and indices of calcium and glucose homeostasis. Univariate and multivariate analysis for associations was performed by regression analysis. The frequencies of the Ala986Ser, Arg990Gly, Gln1011Glu, rs115759455, rs7652589 and rs1501899 alleles in the transplant recipients were 0.88/0.12, 0.94/0.06, 0.97/0.03, 0.95/0.05, 0.63/0.37 and 0.64/0.36 respectively. The CaSR SNP allelic frequencies obeyed the Hardy-Weinberg equilibrium and did not significantly differ from previously reported population cohorts. A significant negative association was revealed between rs7652589 and levels of coronary artery calcifications (odds ratio (OR) = 0.83, $P < 0.05$). Moreover, significant positive associations were demonstrated between rs115759455 and serum phosphate concentrations (OR = 1.14, $P < 0.01$), and Ala986Ser and plasma glucose concentrations (OR = 1.17, $P < 0.05$). Thus, the rs7652589 CaSR SNP was associated with a beneficial effect on intimal calcification within the coronary vessels. Whereas, the rs115759455 and Ala986Ser SNPs were associated with increased levels of serum phosphate and glucose, which are promoters of arterial medial calcification. Our findings indicate novel associations between common CaSR polymorphisms and vascular calcification and glucose homeostasis.
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Testosterone stimulates cholesterol efflux and metabolism in human macrophages via liver X receptor

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Low testosterone is associated with an increased prevalence of cardiovascular (CV) diseases such as atherosclerosis. Testosterone replacement improves several CV risk factors including lowering cholesterol. Liver X receptor α (LXRα) is present in various cell types such as macrophages where it stimulates cholesterol efflux and this ability means LXRα agonists are a potential therapy for atherosclerosis. It was therefore proposed that testosterone acts to reduce the features of atherosclerosis by acting through LXRα. THP-1 macrophages were used, as they express the androgen receptor (AR) and are therefore responsive to testosterone. Cells were treated with 10^{-8} M testosterone (24, 48 and 72 h) either alone or in combination with Flutamide (an AR inhibitor) or LXRα antagonist and gene expression between control and treated cells was assessed by qPCR. The fluorescent cholesterol analogue dehydroergosterol (DHE) was used to directly observe the effect of testosterone on cholesterol efflux. Testosterone significantly increased *LXRα* expression in macrophages. In addition, testosterone increased the expression of genes downstream of LXRα which encode proteins involved in cholesterol efflux and metabolism, including *ABCA1* (*ATP-binding cassette transporter A1*), *APOE* (*apolipoprotein E*), *FAS* (*fatty acid synthase*) and *SREBP1c* (*sterol regulatory element-binding protein 1c*). Blocking LXRα activity inhibited the effect of testosterone, demonstrating testosterone increases *ABCA1*, *APOE*, *FAS* and *SREBP1c* expression by activating LXRα. Testosterone was shown to act via its AR, as blocking the AR inhibited the effect of testosterone on LXRα and LXR-target genes. Testosterone increased the rate of cholesterol efflux from macrophages. We provide evidence that testosterone activates LXRα and acts through this nuclear receptor to control the expression of LXR-target genes to stimulate cholesterol efflux and metabolism. We therefore hypothesize that testosterone exerts its anti-atherogenic effects in part through the activation of LXRα and LXR-target genes.

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FGF21 action on human adipose tissue compromised by reduced βKlotho and FGFR1 expression in type 2 diabetes mellitus

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Background/objectives
Fibroblast growth factor 21 (FGF21) is a potent hormone known to reduce glycaemia and improve insulin resistance with anti-obesity effects. Although mainly secreted in the liver, adipose tissue is considered an important target for its function. Whilst FGF21 has been shown to be expressed in murine adipose tissue, studies in human adipose tissue are lacking. Therefore our aim was to examine the expression of FGF21, FGF21 receptor-1 (FGFR1), βKlotho (co-factor essential for FGF21 function) in human adipose tissue depots and circulating FGF21 in different metabolic states.
Methods
FGF21, FGFR1, and βKlotho mRNA expression was determined in abdominal subcutaneous (AbdSc) and omental (Om) adipose tissue ($n=37$: lean, overweight, obese, type 2 diabetes mellitus (T2DM)). Plasma FGF21 (BMI: 31.5 ± 7.9 kg/m^2; age: 47.1 ± 10.1 years; $n=115$) was measured across metabolic states.
Results
βKlotho mRNA expression was significantly increased in AbdSc adipose tissue from obese (11.2 (mean$\pm$S.E.M.)$0.25\Delta CT$) subjects compared with lean individuals ($12.2\pm0.3\Delta CT$; $P<0.05$). In contrast, βKlotho mRNA expression was significantly reduced in T2DM subjects ($13.5\pm0.3\Delta CT$) compared with lean ($P<0.01$), overweight ($P<0.001$) and obese ($P<0.001$) individuals. FGFR1 mRNA expression was also reduced in T2DM subjects compared with overweight (T2DM: $12.6\pm0.2\Delta CT$ vs overweight: $11.8\pm0.2\Delta CT$; $P<0.01$) and obese (obesity: $11.5\pm0.4\Delta CT$; $P<0.05$) individuals. FGF21 was not expressed in any adipose tissue depot or metabolic state. Circulating FGF21 was significantly raised in T2DM subjects compared with lean ($P<0.001$), overweight ($P<0.001$) and obese ($P<0.05$) subjects.
Conclusions/summary
There was no apparent FGF21 expression in human adipose tissue, whilst βKlotho and FGFR1 were expressed and altered by metabolic state. The raised circulating FGF21 in T2DM together with the reduced expression of βKlotho and FGFR1 in AbdSc adipose tissue suggest that although FGF21 acts on adipose tissue, its action may be compromised by metabolic state. Therefore, the reduced βKlotho and FGFR1 adipose tissue expression, despite increased circulating FGF21, could be a reason for the observed 'FGF21 resistance' in T2DM.

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A component of transcriptional PRC2 complex, enhancer of zest homology, modulates endothelial cell responses to hypoxia and post-ischemic angiogenesis in a mouse model of limb ischemia

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Endothelial cells (ECs) have major role in post-ischemic angiogenesis. Trimethylation of lysine 27 of histone3 (H3K27me3) is a repressive epigenetic mark carried by EZH2 enzyme, the catalytic part of Polycomb complex2 (PRC2, also comprising the suppressor of zeste 12 homolog (Suz12)). We investigated if *in vitro* hypoxia and *in vivo* limb ischemia affect the expression of PRC2 in ECs and mouse limb muscles, respectively. Then, modulation of angiogenic genes by EZH2 and angiogenic responses were assessed using EZH2 inhibitor, 3-deazaneplanocin (DZNep).
Human umbilical vein ECs (HUVECs) were cultured under normoxia or hypoxia (1.2 %O_2, 6–48 h), to mimic ischemia, and treated with DZNep (control: 1% DMSO) or transfected with siRNA against EZH2 (control: scramble oligos). Migration was assessed by scratch assay. The expressions of PRC2 and proangiogenic genes, eNOS, VEGF and VEGF-receptor2, were measured by qPCR. Chromatin-immunoprecipitation coupled with qPCR was performed for EZH2, Suz12 or H3K27me3 at promoters of aforementioned genes. Further, limb ischemia (LI) was used as a mouse model of *in vivo* angiogenesis. CD1 male mice (aged 15 weeks) received DZNep (1.5 mg/kg, i.p. every 2 days) or vehicle 1 day pre-LI. Post-ischemic blood flow (BF) recovery was assessed by colour laser doppler at 30 min and weekly thereafter for 3 weeks. Mice were culled and tissue was snap-frozen or formalin-fixed for analyses.
Hypoxia amplified levels of EZH2 and H3K27me3 in HUVECs. DZNep and EZH2 knock-down reduced expression of EZH2 but not Suz12, and improved migration of HUVECs. Same treatments reversed enrichment of EZH2 or H3K27me3, thus unlocking the expression of above proangiogenic genes. LI has increased the expression of EZH2 and levels of H3K27me3; which were reversed by DZNep. Post-ischaemic BF recovery (weeks 1–3) was increased ($P<0.01$) due to DZNep in line with increased capillary density in the LI-muscles. Therefore, inhibition of EZH2 promotes angiogenesis in both ECs and LI-muscles by enhancing the expression of pro-angiogenic genes.
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The role of hepatic 11β-hydroxysteroid dehydrogenase type 1 in cholesterol homeostasis

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11β-Hydroxysteroid dehydrogenase type one (11β-HSD1) converts inert glucocorticoids to active forms, amplifying intracellular glucocorticoid action. 11β-HSD1 also catalyses the reduction of seven-ketocholesterol (7KC) to 7β-hydroxycholesterol (7βHC). 7KC may inhibit cholesterol biosynthesis (Brown *et al.* 2002). Alteration of cholesterol homeostasis is a major atherosclerotic risk factor. 11β-HSD1 deficiency/inhibition is atheroprotective in animal models, despite only modest changes in plasma cholesterol levels. Here, we have investigated whether 11β-HSD1 influences hepatic cholesterol homeostasis in mice fed fat– or cholesterol-rich diets.
Male mice (5–6-week-old, $n=6$–10/group): 11β-HSD1-deficient (*Hsd11b1*$^{-/-}$), transgenically overexpressing 11β-HSD1 in liver (LOE) or wild-type (WT) were fed chow (C; 11% fat), high fat (HF; 58% fat) or western diet (WD; 38% fat +0.2% cholesterol) for 12 weeks. Liver and fat depots were collected, RNA

extracted and analysed by qPCR. Data (corrected for housekeeping genes) are mean ±S.E.M..

Significantly decreased liver weight was observed for WD-fed *Hsd11b1*$^{-/-}$ mice ($P<0.05$), and reduced mesenteric fat was observed in HF-fed LOE mice compared to WT mice on the same diet ($P<0.05$). Compared to chow, WD decreased hepatic levels of mRNAs encoding SREBP2, HMG-CoA-reductase and HMG-CoA-synthase in WT mice as predicted, and in LOE mice ($P<0.001$). Hepatic LXRα mRNA was unaffected by diet in WT and *Hsd11b1*$^{-/-}$ mice (and did not differ in chow-fed mice between genotypes), but was increased in WD-fed LOE mice (WT, 100±3.46 vs LOE, 178.48±6.21, $P<0.05$), as were the LXRα targets, *Abgc5/8* (*Abcg5*: WT, 100±4.65 vs LOE, 242.13±9.91, $P<0.001$; *Abcg8*: WT, 100±3.23 vs LOE, 167.77±5.49, $P<0.01$).

These data do not support a role for hepatic 11β-HSD1 in *de novo* cholesterol synthesis. However, increased hepatic *Abcg5/8* expression in WD-fed LOE mice suggests hepatic 11β-HSD1 promotes sterol efflux into the intestinal and biliary lumen, possibly meditated through higher *Lxrα* expression. This suggests a role for hepatic 11β-HSD1 in promoting biliary cholesterol secretion.

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P182

Vitamin B12 and folate imbalance induces cholesterol synthesis and endoplasmic reticulum stress in human adipocytes

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Adipose tissue (AT) plays a central role in integrating energy metabolism and glucose homeostasis. It is the major site of fatty acid storage as triglycerides and is body's largest cholesterol pool. In AT cholesterol is mostly found in its free, non-esterified form. There is accumulating evidence that cholesterol imbalance in AT is closely associated with adipocyte dysfunction and obesity-mediated metabolic complications, including low levels of high-density lipoprotein cholesterol and insulin resistance. Low levels of methyl donor S-adenosylmethionine (SAMe) has been shown to activate SREBP-1 and lipogenesis. Similarly, vitamins B12 and folate regulate the levels of SAMe and homocysteine. As homocysteine has been shown to induce ER stress, we investigated the effect of B12/folate on cholesterol synthesis and ER stress in adipocytes.

Human pre-adipocyte cell line CHUB-S7 was differentiated in various B12/folate concentrations: i) control: (B12 500 nM, folate-6 μM); ii) normal B12/high folate: (B12 500 nM, folate-15, 30, 60 μM). iii) Low B12/high folate: (B12 0.15 nM, folate-6, 15, 30, 60 μM) and iv) No B12/high folate: (B12 0 nM, folate-0, 30, 60 μM). DNA, RNA and protein was extracted from the differentiated adipocytes and used for investigation of cholesterol biosynthesis pathway using microarray, real-time PCR and western blotting. Conditioned media was used for homocysteine analysis by HPLC.

Microarray analysis led to identification of cholesterol biosynthesis and ER stress pathways, altered due to B12/folate imbalance. Validation by real-time-PCR confirmed that compared to normal B12/folate levels, the genes involved in cholesterol biosynthesis, regulation and ER stress were up-regulated in both B12 deficient conditions ($P<0.05$). Protein expression of SREBP-1 and SREBP-2 were also increased in the same conditions including levels of total cholesterol and homocysteine (all $P<0.05$).

Our data provides a novel mechanism that adipocytes subjected to inappropriate levels of B12/folate exhibit increased cholesterol biosynthesis and ER stress, thus potentially predisposing adipocyte dysfunction.

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P183

The effects of *Syzygium aromaticum*-derived oleanolic acid on reactive oxygen species in the heart, liver and kidney of STZ-induced diabetic rats

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The onset of diabetic complications is attributed to sustained hyperglycemia which triggers the generation of free radicals and oxidative-related damage in the retina, renal glomerulus and peripheral nerves. Recent studies report that intense glycaemic control by the subcutaneous administration of insulin cannot completely restore the balance between reactive oxygen species and antioxidants. Preliminary studies in our laboratory indicate that transdermally delivered *Syzygium aromaticum*-derived oleanolic acid (OA) has the ability to lower blood glucose in experimental diabetes mellitus due to the sustained release of the triterpene. However, no work has been done to determine the effects of OA on reactive oxygen species (ROS). Research has indicated that some bioactive compounds such as flavonoids and tannins have antioxidant properties. Accordingly, this study was designed to investigate and evaluate the effects of *S. aromaticum*-derived OA on ROS levels. The acute effects of OA were evaluated on malondialdehyde (MDA) and glutathione (GSH) concentrations in STZ-induced diabetic rats following a glucose load after an 18-h fast. Rats administered pectin-free OA or transdermally delivered insulin acted as untreated and treated positive controls, respectively, while non-diabetic rats served as absolute controls. The transdermal patches were applied for 6 h, thereafter the animals were sacrificed. The heart, liver and kidney were collected for ROS biomarkers (MDA) and antioxidants (GSH) analysis. MDA levels were significantly reduced in the heart (233.0±0.1 vs 47.0±0.1 nmol/l) and liver (233.0±0.1 vs 130.0±0.1 nmol/l). Interestingly, GSH levels were also significantly increased in the heart (196.0±0.4 vs 313.0±0.5 nmol/l). These results suggest that *S. aromaticum*-derived OA is potentially effective in ameliorating the oxidative stress observed in diabetes mellitus in the heart. It can therefore, be concluded that OA is a potential drug for diabetes mellitus that would not only lower blood glucose but also can avert complications that arise due to oxidative stress.

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P184

Effects of vitamin D supplementation on blood pressure, glucocorticoids and cardiovascular risk markers in healthy subjects

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Background

Recently, vitamin D has received an enormous attention, primarily at the public health level due to its numerous biological effects. It has been suggested that vitamin D deficiency may adversely affect blood pressure and the cardiovascular system. The aim of this project was to determine the possible effects and association between vitamin D intake, blood pressure, glucocorticoids and CVD risk factors.

Methods

Two pilot studies have been carried out; one using short-duration repeated measure ($n=20$) and the second was a randomised parallel controlled design ($n=38$). Healthy volunteers from Queen Margaret University were recruited. Each participant was asked to consume 20 μg (800 IU/day) of vitamin D3 supplement per day for 14 days (1st study), and vitamin D or placebo for 28 days (2nd study). Three readings of systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse wave velocity (PWV) were recorded at baseline and at the end of the intervention. Blood (glucose and lipids), saliva and 24h urine (glucocorticoids) samples were obtained. Diet dairies and lifestyle questionnaires were also monitored through out the study.

Results

Vitamin D intake increased significantly in both studies ($P<0.001$) and thus indicating compliance. Mean PWV showed a significant decrease of 0.74 m/s ($P=0.017$), with a negative correlation with vitamin D intake ($r=-0.43$). There was also a significant decrease in mean SBP (115.3±13.1–110.9±10.8, $P=0.035$) and DBP (73.6±10.6–69.8±9.1, $P=0.04$). There was no significant change in BMI between baseline and final measurements ($P=0.527$). No significant differences were found between the groups in total, LDL cholesterol, triglycerides and glucose except HDL increased following 4 weeks of D intake;

from 0.92±0.12–1.24±0.35 mmole/l ($P=0.025$). There was no effect on salivary cortisol but cortisone increased (0900 h: 7.33±2.6–9.98±5.3 nM, $P=0.04$). Urinary free cortisol/cortisone ratio was reduced (1.91±0.75–1.22±0.53, $P=0.015$).

Conclusion

The results suggest that moderate intake of vitamin D can influence salivary and urinary glucocorticoids, attenuate BP, improve cardiovascular markers and might be beneficial to prevent contemporary diseases. Further studies to elucidate the effects of the 'sunshine' vitamin, particularly in relation to CVD risk factors would be justified.

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P185

Knockdown of brain 11β-HSD1 does not lower body weight or improve insulin sensitivity

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Glucocorticoids act on several major neuropeptide networks in the hypothalamus which are important for regulation of energy balance and insulin sensitivity. Active glucocorticoids (cortisol/corticosterone in humans/rodents) can be regenerated from their inactive forms by the enzyme 11β-hydroxysteroid dehydrogenase type one (11β-HSD1) which is expressed in the hypothalamus. Therefore we decreased 11β-HSD1 expression in the brain to investigate the role of regenerated corticosterone on metabolic parameters.

Knock-down of 11β-HSD1 in the CNS (BKO) using a Nestin-*Cre* promoter led to >90% inhibition of 11β-HSD1 activity and expression in the brain. Twelve-week-old BKO, 11β-HSD1 conditional (floxed) and Nestin-*Cre* mice were placed on high fat diet (HFD) for 12 weeks. The BKOs were lighter than the floxed mice, but similar to the Nestin-*Cre* mice. There was no difference in % body fat measured by dual energy X-ray absorptiometry (DXA; Nestin-*Cre*: 41.5±1.7 vs BKO: 39.4±2.6 vs floxed: 38.7±6.2) or fat distribution. BKO had a reduced food intake compared to floxed animals, but higher food intake than Nestin-*Cre*. Although decreased glucocorticoids in the hypothalamus would be expected to improve insulin sensitivity, BKO and Nestin-*Cre* mice had similar insulin sensitivity measured during an oral glucose tolerance test (OGTT) with the floxed mice tending to be less insulin sensitive. Glucose responses were not different between any of the three strains. Reduction of glucocorticoids in the brain, may be expected to upregulate the hypothalamic-pituitary-adrenal axis through reduction in negative feedback, however, there was no difference in adrenal gland weight between the three strains (BKO: 2.3±0.6 vs nestin-*Cre*: 2.2±0.4 vs floxed: 2.6±0.7 mg).

In summary, 11β-HSD1 knockdown in the brain does not improve the metabolic effects of HFD. This suggests that 11ßHSD1 inhibition in brain is unlikely to be a primary driver of efficacy in the search for11β-HSD1 inhibitors to treat type two diabetes and obesity.

Declaration of interest

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P186

C-type natriuretic peptide down regulates interferon γ mediated pro-inflammatory gene expression in human endothelium

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Cardiovascular diseases account for more deaths in the Western world than from any other cause. Atherosclerosis has a chronic inflammatory component involving Th1 pro-inflammatory cytokines such as IFN-γ, which is known to induce endothelial cell inflammatory responses. CNP, acting via its receptors to elevate intracellular cGMP, is produced by endothelium and endocardium and is upregulated in atherosclerosis. It is believed to be protective yet its role in vascular inflammation is poorly understood. The aim of this study was to investigate effects of CNP on human endothelial cell inflammatory responses following IFN-γ stimulation. HUVECs were treated with either IFN-γ (10 ng/ml) or CNP (100 nm), or both in combination, followed by analysis by flow cytometry for expression of MHC class 1 and ICAM-1. In experiments, CNP was substituted by the cGMP donor 8-Bromoguanosine 3′, 5′-cyclic monophosphate. To determine whether CNP also modulates the anti-microbial effects of IFN-γ in HUVEC, expression of indolamine deoxygenase (IDO) was measured by RT-PCR. To determine whether CNP directly affects the signalling pathways activated by IFN-γ the phosphorylation of STAT-1b was analysed by western blotting and immunodetection. IFN-γ significantly increased expression of both MHC class 1 and ICAM-1, which was significantly inhibited by CNP or 8-Br-cGMP. CNP also reduced IFN-γ mediated phosphorylation of STAT-1b and total levels of STAT-1b and enhanced IFN-γ mediated expression of mRNA for IDO. CNP downmodulated IFN-γ induced pro-inflammatory gene expression in human endothelial cells via a cGMP-mediated pathway, as well as upregulating IFN-γ mediated expression of anti-microbial genes, which are considered to be protective in a chronic inflammatory response. Thus, CNP has a protective role in vascular inflammation and novel therapeutic strategies for CVD based on upregulation of endothelial CNP expression could reduce chronic EC inflammation.

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P187

Acute effects of co-infusion of peptide YY (3–36) and glucagon-like peptide-1 on insulin secretion and insulin sensitivity

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The amelioration of type 2 diabetes and sustained weight loss after bariatric surgery are thought to be due to elevated circulating levels of the gut hormones peptide $YY_{3–36}$ ($PYY_{3–36}$) and glucagon-like peptide-1 (GLP-1). GLP-1 augments the insulin response to an oral glucose load. $PYY_{3–36}$ has appetite-inhibitory effects and contributes to longer-term weight loss. Rodent studies provide conflicting data regarding the effects of PYY on glucose homeostasis: PYY may enhance insulin-mediated glucose disposal, but *in vitro* evidence suggests that the activation of Y1-receptors (which mediate the effects of PYY on pancreatic islet cells) may inhibit insulin release. No study to date has examined the effects of PYY on glucose homeostasis, either alone or in combination with GLP-1, in humans.

Hypothesis

GLP-1 will exert a beneficial effect on glucose homeostasis in healthy, overweight human volunteers, and co-administration with $PYY_{3–36}$ will not attenuate this response.

Methods

Fourteen overweight healthy volunteers were studied in a single-blinded crossover fashion. They were randomised to receive four infusions: i) vehicle; ii) GLP-1 at 0.2 pmol/kg per min; iii) $PYY_{3–36}$ at 0.15 pmol/kg per min; and iv) co-infusion of GLP-1 plus $PYY_{3–36}$ at the above doses. During each intervention, a frequently sampled intravenous glucose tolerance test was performed to assess acute insulin response to glucose (AIRg), and insulin sensitivity (Si), using minimal modeling.

Results

AIRg was significantly increased by infusion of GLP-1 alone, compared to all other interventions ($P<0.05$). No acute effect on Si was noted with any of the interventions. The disposition index (AIRg×Si) was significantly greater following administration of GLP-1 alone than with any other intervention ($P<0.05$).

Conclusion

GLP-1 infusion increases the AIRg in healthy, overweight humans. In contrast, PYY infusion had no effect on AIRg and neither hormone, alone or in combination, was shown to enhance Si in the acute setting.

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P188

Human abdominal subcutaneous adipocytes as an active source of LpPLA2, influenced by fat depot and metabolic state, with LpPLA2 converting LDL into more potent atherogenic Ox-LDL, *in vitro*

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Lipoprotein-associated phospholipase A2 (LpPLA2) is a member of the phospholipaseA2 super family of enzymes, and is upregulated in arterial inflammation, obesity and cardiovascular disease. The other isoforms, iPLA2 and cPLA2, appear to contribute to inflammation through production of lipid mediators. The role of PLA2 in human adipose tissue (AT) is unclear, therefore we sought to i) characterise PLA2 isoforms in lean, obese, T2DM abdominal subcutaneous (AbdSc) and omental (Om) AT ii) evaluate the role of lipids and inflammatory markers on circulating LpPLA2 levels and iii) determine the *in vitro* regulation of LpPLA2 in human adipocytes.

AT and sera from lean, overweight, obese and T2DM subjects were taken. PLA2 gene expression was determined by microarray, RT-qPCR and Western Blot. Association between circulating LpPLA2 and metabolic parameters were investigated. The human adipocyte cell line, Chub-S7, was used to assess the effects of oxidized LDL on PLA2 expression.

LpPLA2 mRNA levels were higher in AbdSc AT than Om AT in obesity by twofold ($P<0.05$). The cPLA2 protein expression was increased with obesity in AbdSc AT ($P<0.01$). T2DM showed increased LpPLA2 mRNA levels in AbdSc ($P<0.001$) and OmAT ($P<0.01$). Serum LpPLA2 showed positive correlations with cholesterol, TG, LDL, endotoxin and oxidized LDL (Ox-LDL) ($P<0.001$) in non-diabetic subjects and with Ox-LDL ($P<0.001$), LDL ($P<0.01$) and cholesterol ($P<0.05$) in T2DM. In differentiated pre-adipocytes, activation of LpPLA2 protein expression was noted in response to LDL and Ox-LDL ($P<0.001$).

The adipocyte appears to be an active source of LpPLA2, altered by fat depot and metabolic state, with LpPLA2 protein expression being induced by LDL and Ox-LDL *in vitro*. As such, increased LpPLA2 protein from the adipocyte in obesity and/or T2DM may contribute to raise circulating Ox-LDL, which promotes further inflammation and atherosclerotic risk. Taken together, LpPLA2 in the adipocyte and AT represents an important therapeutic target to reduce inflammation, atherosclerotic risk and development of metabolic complications.

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P189

The role of glucocorticoid metabolism in bile acid homeostasis

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Background and aims

Bile acids regulate cholesterol metabolism and the digestive system. They are conserved through enterohepatic circulation, a glucocorticoid-modulated process. We investigated whether the regeneration of active glucocorticoid by 11β-hydroxysteroid dehydrogenase type one (11β-HSD1) affects bile acid release and enterohepatic transport after re-feeding.

Methods

Bile acid turnover was assessed in global ($Hsd11b11^{-/-}$), liver-specific knockout ($Hsd11b1^{LKO/LKO}$) mice as well as mice with liver-specific over-expression of 11β-HSD1 ($Hsd11b1^{LOE/LOE}$), compared with their respective genetic controls (C57Bl/6 and 'floxed' $Hsd11b1^{F/F}$ littermates). Adult (12 week) male, chow-fed mice ($n=8$/group), were either fasted for 4 h or fasted for 4 h then re-fed 4 h. Serum bile acids were measured spectrophotometrically and corticosterone by ELISA. Body and tissue weights, food and fluid intake were recorded.

Results

Serum bile acids remained high in re-fed $Hsd11b1^{-/-}$ mice (fasted, 30+9 nM vs refed, 24+12 nM) in contrast to C57BL/6 mice (fasted, 3.8+1.3 nM vs refed, 62.5+7.6 nM, $P<0.0001$). $Hsd11b1^{LKO/LKO}$ mice replicated all the effects seen in $Hsd111b1^{-/-}$ mice whilst $Hsd11b1^{LOE/LOE}$ was identical to C57BL/6. $Hsd11b1^{-/-}$ mice failed to show the normal gall bladder weight change upon re-feeding compared to C57BL/6 (C57BL/6: fasted 100+6% vs re-fed, 49.6%+8, $P<0.0001$; $Hsd11b1^{-/-}$: fasted, 96+6.5% vs refed 92+7.8%). $Hsd11b1^{-/-}$ mice showed reduced food and water intake following re-feeding (Food: C57BL/6 100+7%, *vs* $Hsd11b1^{-/-}$ 72.3+14% $P<0.05$. Water: C57BL/6 100+15% *vs* $Hsd11b1^{-/-}$ 63+4%, $P<0.001$). Basal, noon and evening corticosterone levels showed a normal circadian rhythm but did not differ between genotypes.

Conclusions

These data suggest that hepatic 11β-HSD1 deficiency reduces enterohepatic circulation of bile acids by preventing gall bladder emptying. Thus inhibition of hepatic11β-HSD1 may lead to cholestasis. Whether acute changes in food and water intake are directly caused by 11β-HSD1 activity or are secondary to changes in bile acid homeostasis is not clear.

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P190

Testosterone differentially regulates lipid and glucose metabolism in visceral and subcutaneous fat in the testicular feminised mouse

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Objectives

Testosterone deficiency is common in obese men with type 2 diabetes. Testosterone replacement therapy (TRT) improves insulin resistance, glycaemic control and cholesterol in hypogonadal men, and TRT reduces body fat mass. Adipose tissue plays a major role in glucose homeostasis and insulin sensitivity through the regulation of lipid and glucose metabolism. There are functional differences between subcutaneous and visceral adipose tissue. This study investigates the expression of key targets involved in lipid and glucose homeostasis in visceral and subcutaneous fat depots of the testicular feminised (Tfm) mouse, which exhibit non-functional androgen receptors and low circulating levels of testosterone.

Methods

Tfm mice were fed a high-cholesterol diet ad libitum for 28 weeks and received either TRT (intramuscular mixed testosterone esters) or placebo (saline) and were compared to wild-type littermates. Visceral and subcutaneous abdominal adipose tissue was collected and relative concentrations of mRNA was analysed by qPCR for the expression of gene targets involved in lipid metabolism (acetyl coA carboxylase, ACC; fatty acid synthase, FAS; hormone sensitive lipase, Lipe; lipoprotein lipase, LPL), cholesterol efflux (apolipoprotein E, ApoE; ATP-binding cassette transporter A1, ABC-A1), glucose control (glucose transporter 4, Glut4; hexokinase 2, HK2; phosphofructokinase, PFK) and master regulators of lipid and glucose metabolism (peroxisome proliferator-activated receptor-α, PPARα; PPARγ; liver X receptor, LXR; sterol regulatory element binding protein-1, Srebp-1; Srepb-2).

Results

Compared to littermates, Tfm mice had significantly lower subcutaneous mRNA expression of LPL, ApoE, Glut4 HK2, PFK, PPARα, PPARγ, LXR, Srebp-1 and Srebp-2 ($P\leq0.05$, $n=9$) and increased Lipe. In visceral fat, only PPARγ was significantly reduced ($P<0.001$, $n=9$). TRT increased the expression of LXR, ApoE and Srebp-1 in subcutaneous fat ($P\leq0.05$, $n=9$), showed a trend towards increased srebp-2 ($P=0.07$) and HK2 ($P=0.09$) and decreased Lipe. However, TRT had no significant effect on the expression of any targets in visceral fat.

Discussion

Subcutaneous fat is intrinsically different from visceral fat and testosterone acts differentially on targets of glucose and lipid metabolism in these depots to potentially influence whole-body metabolism.

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P191

Novel inositol pyrophosphate and insulin sensitivity in response to muscle contraction in glucose intolerant humans

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Peripheral insulin resistance is a major defect associated with glucose intolerance and type two diabetes. Yet the mechanism(s) responsible for this defect remain to be determined. Inositol pyrophosphate (IP7) is formed via the enzyme IP6K1 from IP6. The inhibition of Akt, and the potential decrease in insulin signalling, through the binding of IP7 to the Akt PH domain represents an exciting research area.

Firstly, we will characterise this mechanism in humans for the first time. Secondly, we will test for correlations of IP6K1 activity with *in vivo* measures of insulin sensitivity (SI2*) and glucose effectiveness (SG2*). Thirdly, using a stimulus that is known to improve insulin sensitivity (i.e. muscle contraction), we will test if this pathway can be altered following exercise in glucose intolerant individuals (mean (S.D.) age 52.2 (5.3) years; BMI 30.7 (6.4); HOMA-IR 3.4 (1.2); fasting blood glucose 5.0 (0.5) mmol/l) ($n=4$). Analysis of our data is nearing completion but preliminary results show that area under the curve for glucose (AUC_{Glu}) is lower immediately post high-intensity intermittent exercise (IE) (resting control; 1276 (60) vs IE; 1197 (115) mmol/l per min) while acute insulin response to glucose (AIR_{glu}) is elevated following IE at 745 (98) compared to 391 (104) μU/ml per min for the control trial. These data suggest that this form of exercise improves glucose tolerance. We now wish to determine if these changes are reflected in activity levels and protein content of IP6K1 and IP6/IP7, respectively in our complete data set ($n=15$). These samples have been collected using standard muscle biopsy methodologies and will be fully analysed and presented in our final abstract.

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Augurin stimulates food intake in male Wistar rats

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Augurin is a putative neuropeptide product of the esophageal cancer-related gene-4. We have previously demonstrated intra-cerebroventricular (ICV) and intra-paraventricular nucleus (iPVN) administration of augurin (71–148) stimulates the hypothalamo-pituitary-adrenal axis in male Wistar rats. This effect was dependent on the release of CRH. Since CRH is known to inhibit feeding, we hypothesised augurin (71–148) would have similar actions. We therefore investigated the effects of augurin (71–148) on light and dark phase feeding in male Wistar rats. I.c.v. administration of augurin had no significant effects on feeding. In contrast, iPVN administration of augurin (1 and 2 nmol) to *ad libitum* fed rats during early dark phase (1900–2000 h) significantly increased food intake 0–1 h after injection (0–1 hour food intake/g: vehicle 4.8 ± 0.3; augurin 1 nmol 6.8 ± 1.0, $P<0.05$; 2 nmol 7.3 ± 0.4, $P<0.01$). Similarly, iPVN administration of augurin (0.5 nmol, 1 and 2 nmol) to ad libitum fed rats during early light phase (0900–1000 h) significantly increased food intake 0–1 hours after injection (0–1 hour food intake/g: vehicle 0.7 ± 0.1; augurin 0.5 nmol 1.8 ± 0.4, $P<0.05$; 1 nmol 2.1 ± 0.4, $P<0.05$; 2 nmol 2.1 ± 0.3, $P<0.01$).

These studies are the first to suggest a role for augurin in the stimulation of feeding. The effect may be mediated via the hypothalamic paraventricular nucleus. Further work is now needed to determine the precise mechanisms behind these effects and whether augurin has an important role in feeding behaviour.

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P194

Intracellular delivery of therapeutic siRNA via an antennapedia-double stranded RNA binding domain fusion protein as a novel strategy for PTP1B translation attenuation in type 2 diabetes

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Type 2 diabetes is a metabolic disorder characterised by insulin resistance and is associated with diminished signalling through the insulin receptor. Protein tyrosine phosphatase 1B (PTP1B), encoded by the PTPN1 gene, has been shown to attenuate insulin's actions by de-phosphorylating the activated insulin receptor and downstream signalling components such as IRS1. Several studies have highlighted PTP1B as a potential therapeutic target for type 2 diabetes and obesity; however, small molecule drug discovery efforts have proved to be challenging due to issues with achieving selectivity and bioavailability. Inhibition of PTP1B expression via the RNAi pathway represents an alternative therapeutic approach; however, due to their large size and charge these molecules cannot enter cells unaided. Here we aim to characterize a novel siRNA delivery method using a fusion protein containing the *Drosophila melanogaster* Antennapedia homeoprotein (AntpHD) transduction domain, which has the innate ability to cross cell membranes, and the double-stranded RNA binding domain (dsRBM1) from human protein kinase R, which binds dsRNA with high avidity. Fusion proteins consisting of either the AntpHD, or its third helix, Penetratin, fused to dsRBM1 will be tested as a means to deliver PTP1B siRNA into HepG2 cells with the aim of knocking down PTP1B and consequently potentiating insulin-mediated effects. Multiple constructs have been successfully cloned, expressed and purified using an *E. coli* expression system and characterized by SDS–PAGE and mass spectrometry. Indirect immunofluorescence studies using a myc-tagged construct have demonstrated the ability of the proteins to cross the cell membrane and localize within cytoplasmic vesicles after 2 and 24 h treatment, at 1 μM concentrations, while higher concentrations (10 μM), resulted in time-dependent peri-membrane aggregation with some intracellular localization in $\geq 50\%$ of cells. Ongoing experiments are underway to assess the effectiveness of this method as an approach to knockdown PTP1B and potentiate insulin signalling.

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P195

Replication of genome wide association-validated loci for type 2 diabetes mellitus in the Saudi Arabian population

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Background

Previous genome wide association studies in Caucasian and South Asian populations have identified over 35 loci for type 2 diabetes mellitus (T2DM) risk. However, little is known about the contribution of these loci in T2DM from a Saudi Arabian population. In this study we investigated for the first time, the association of 38 previously identified T2DM risk loci (32 loci from Caucasian and six loci from South Asian populations) in 1166 T2DM patients and 1235 healthy controls from Saudi Arabia.

Methods
All DNA samples from cases and controls were genotyped for 38 SNPs using the KASPar method (KBioscience, Hoddesdon UK).
Results
Common genetic variants (in or near *WFS1, JAZF1, CDKN2A/B, TCF7L2, KCNQ1, HNF4A,* and *DUSP9*) showed significant ($P<0.05$) associations with T2DM in our study population. The effect sizes of these loci were comparable to those previously identified with the exception of *HNF4A* which showed evidence of heterogeneity with a trend for larger effect size in our study population (OR, 95% CI; 1.27, 1.07–1.51) compared to that reported in South Asian populations (1.09, 1.06-1.12, $I^2=65.9$). Analysis of risk allele scores (RAS) defined by the T2DM-associated loci showed that subjects in the top 20% of the RAS distribution ($n=480$) had 2.5 fold increase in disease risk as compared to those in the lower 20% ($n=480$; $P=9.5\times10^{-12}$). RAS were also associated with fasting glucose level ($\beta=0.12$; $P=2.2\times10^{-9}$) but not with BMI ($P=0.19$).
Conclusion
In conclusion we have shown for the first time that variants at *WFS1, JAZF1, CDKN2A/B, TCF7L2, KCNQ1, HNF4A, and DUSP9* are associated with T2DM in the Saudi population but further larger studies will be required to confirm these findings in other Middle Eastern populations with high T2DM prevalence and to identify other T2DM-susceptibility loci.

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P196

Cellular consequences for insulin signal transduction of the naturally occurring AKT2 p.Glu17Lys mutation

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Introduction
We have reported a syndrome of hypoketotic hypoglycaemia due to the *de novo* p.Glu17Lys mutation in AKT2, a serine threonine kinase central to insulin signalling. We aimed to evaluate further the physiological and cellular consequences of the p.Glu17Lys mutation.
Results
Two previously reported 17-year-old men heterozygous for the AKT2 p.Glu17Lys mutation, each of whom had experienced severe fasting hypoglycaemia in early life, were re-evaluated. Both had 37% body fat and normal levels of hepatic triglyceride and fasting *de novo* lipogenesis. In patient one, who has a family history of obesity-related insulin resistance (IR), blood glucose remained normal overnight with concomitantly low plasma insulin levels, and a normal increase in free fatty acids (FFA). In patient two hypoglycaemia with undetectable insulin occurred after 2 h, and recurred after oral glucose. The FFA profile in this patient was nearly flat. Oral glucose tolerance testing showed no evidence of diabetes in either patient. In primary fibroblasts from patient one low level basal phosphorylation of AKT and its substrates p70 S6 kinase and GSK3α/β was seen, but, surprisingly, their peak phosphorylation after insulin or EGF stimulation was attenuated. In 3T3-L1 cells conditionally overexpressing mutant but not wildtype AKT2 nuclear exclusion of FoxO1a was seen, but no difference in phosphorylation of AKT2 nor in basal or insulin-stimulated deoxyglucose uptake was observed.
Conclusion
The AKT2 p.Glu17Lys mutation produces mild constitutive activation of signalling in primary fibroblasts. In the 3T3-L1 model this is sufficient to suppress FoxO1a activity but not to activate glucose transport, consistent with the low glucose required to maintain normoglycaemia. The clinical importance of the attenuated peak cellular response to ligand is unclear, while the 'autocorrection' of one patient with a family history of obesity-related IR may offer insights into the level at which insulin action is impaired in prevalent disease.
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P197

DPPIV and macronutrients regulate the expression of PYY3–36 in human gut epithelial cells

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The enzyme dipeptidyl peptidase-IV (DPP-IV) is expressed by gut epithelial cells. DPP-IV cleaves X-proline dipeptides from the N-terminus of polypeptide hormones including GLP-1, GIP and PYY. DPP-IV causes a deactivation of the incretin hormones GLP-1 and GIP, whereas conversely, by converting PYY1–36 to PYY3–36 it allows specific activation of the Y_2 receptors in the arcuate nucleus of the hypothalamus leading to hypophagia. Infused PYY3–36 has been shown to reduce food intake in healthy and obese humans by 30%. Furthermore, high protein meals have been shown to significantly increase plasma PYY3–36 levels compared to carbohydrate or fat. The role of DPPIV enzyme expression in intestinal cells or the role of individual amino acids on the up regulation of PYY has not been investigated.
Using the Caco-2 human intestinal epithelial cells we investigated whether the effect of specific macronutrients on PYY and DPPIV gene expression. Cells were stimulated for 24 h with 1.59 mM L-leucine, 2.39 mM L-arginine, L-Leu+L-Arg, non essential amino acids (NEAA)×10 concentration and 20 nM glucose. The expression of DPP-IV and PYY1–36 genes was determined by SYBR-green real time PCR.
Our results showed no change to the DPP-IV and PYY1–36 gene expression on stimulation with either L-Leu or L-Arg in isolation. However, addition of both L-Leu+L-Arg in combination resulted in a 1.5-fold induction of DPP-IV ($P=0.017$) gene and 1.8-fold up-regulation of PYY ($P=0.019$) gene expression. Glucose treatment also caused a 1.4 up-regulation of DPPIV ($P=0.041$) and a 3.3 up-regulation of PYY1 ($P<0.0001$) genes. These data show for the first time that PYY gene is expressed in the human gut epithelial cell line – Caco-2 and that the increase in plasma PYY3–36 may therefore be a result of increased expression of both PYY and DPPIV genes in human intestinal cells.
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P198

Inflammatory markers in diabetic foot and impact of vitamin D deficiency

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Objective
i) To evaluate plasma levels of IL6, adiponectin and resistin in subjects with diabetic foot in comparison with subjects without foot complications. ii) To assess the impact of vitamin D status on the levels of above inflammatory markers.
Methods
A total 100 diabetic foot cases and 100 diabetic controls were recruited in the study. Serum level of 25OH vitamin D was estimated from the cases & controls by RIA. Serum IL6, adiponectin and resistin were assayed by ELISA. Data were analyzed using online graphpad quickcalc software and $P<0.05$ was considered statistically significant.
Results
Mean age of the study population was 54.3 ± 12.4 years (male:female=68:32). Mean age of the controls was 52.5 ± 13.6 (male:female=60:40). HbA1c was comparable (10.3 vs 10.9%). Diabetic foot cases were having lower vitamin D status (16.1 ± 16.0 ng/ml) than the diabetic controls (19.8 ± 14.1 ng/ml). Prevalance of vitamin D deficiency was higher in cases than controls (62 vs 57%). Females outnumbered males in terms of prevalence of vitamin D deficiency (22/32 females (68%) vs 40/68 (58%) males in cases and 25/ 40 females (62%) vs 32/60 (53%) males in control group). Severity of vitamin D deficiency (<10 ng/ml) was higher in cases (48.2%) than controls (26.2%).
IL6 level was higher in cases (128.3 pg/ml) than the controls (63.8 pg/ml) (*P*-0.01). Similarly lower median plasma levels of adiponectin (7.7 vs 8.4 µg/ml) and higher median plasma levels of resistin (3.8 vs 3.6 ng/ml) were observed in cases ($P<0.05$). No significant difference was observed in the levels of these markers between male and female study participants in both the groups. Patients under vitamin D deficient group (<30 ng/ml) demonstrated higher IL6 (130.8 vs 100.0 pg/ml), higher resistin (3.9 vs 3.6 ng/ml) and lower adiponectin (7.6 vs 8.3 µg/ml) levels compared to vitamin D sufficient (≥ 30 ng/ml) group in diabetic foot ($P<0.05$).

Discussion
Immuno-regulatory role of vitamin D is well established. Diabetic foot infections reflect the immune-compromised state of the patients and therefore it is speculative that vitamin D deficiency is more common and severe in diabetic foot. Our study demonstrated that diabetic subjects with diabetic foot showed in comparison with diabetics without diabetic foot higher IL6 and resistin plasma levels and lower adiponectin plasma levels. Hypovitaminosis D is more prevalent in patients with diabetic foot and Vitamin D deficiency is more severe in patients with diabetic foot infections. The levels of the above markers are more in diabetic foot patients with vitamin D deficiency.
Conclusion
Assumption is made that Vitamin D deficiency enhances inflammatory response in addition to hyperglycemia, in diabetic foot.

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P199

The possible involvement of the receptor for advanced glycation end products in vascular senescence in diabetes

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Introduction
RAGE activation plays a pivotal role in the pathogenesis of diabetic vasculopathy, however the signal transduction mechanism downstream of RAGE is not fully understood. Yoshimoto et al. previously established the RAGE-overexpressed vascular smooth muscle cell line, RAGE-A10, and found that its slow growth rate and increased monocyte chemotactic protein-one (MCP-1) mRNA expression were analogous to the phenomenon of senescence. Senescent cells display characteristic morphology, including increased senescence-associated ß-galactosidase (SA-ßG) activity, increased proinflammatory status and a slow growth rate.
Aim
This study was undertaken to investigate the possible cellular senescence of RAGE-A10 and determine its potential signal transduction mechanism(s).
Method
Four rat VSMC cell lines (A10) expressing human RAGE and control cell lines pMX-A10 were cultured. SAßG staining cells were counted by light microscopy. Enzyme-linked immunosorbent assay measured MCP-1 pro-inflammatory protein levels. RAGE cells were treated for 72 h with ligand S100B; NAD(P)H oxidase inhibitor, apocynin; inhibitor of IκB phosphorylation, Bay11074; MEK1 inhibitor, U0126; and Src inhibitor, PP2, all known to be involved in diabetic vasculopathy, Flow cytometry examined cell cycle distribution.
Results
RAGE-A10 cells demonstrated increased SA-ßG activity, significantly increased protein expression of MCP-1 ($P<0.05$) with ligand stimulation and a slower growth rate, due to delayed G1/S phase cell cycle progression. NF-κB, MAPK and non-receptor tyrosine kinase Src involvement in ligand-induced MCP-1 expression in RAGE-A10 was clearly shown. However increased SA-ßG activity and reduced growth rate appear to be mediated by a ligand-independent signaling pathway.
Conclusion
Our findings demonstrate that RAGE-overexpressed A10 cell lines exhibit characteristic hallmarks of senescence, represented by increased SA-ßG activity, increased proinflammatory status and a slower rate of cell proliferation, via an as yet unknown signal transduction pathway(s). Further investigation into mechanisms downstream of RAGE may provide clearer insight into potential therapeutic targets against RAGE and senescence in the treatment of diabetic vasculopathy.

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P200

Effects of *Syzygium aromaticum*-derived oleanolic acid administration on postprandial glucose concentration and key intestinal carbohydrate hydrolyzing enzymes of streptozotocin-induced diabetic rats

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The magnitude and duration of postprandial blood glucose elevations due to hydrolysis of carbohydrates are major risk factors of diabetes and coronary heart diseases. Inhibition of the key carbohydrate hydrolyzing enzymes in the small intestine suppresses postprandial blood glucose peaks and reduces chronic vascular complications in diabetic subjects. Recent reports indicate that *Syzygium spp*-derived oleanolic acid (OA) inhibits glucose transport in the small intestine, but its effects on postprandial hyperglycaemia and key carbohydrate hydrolyzing enzymes remain unanswered. Accordingly, postprandial blood glucose variation was evaluated in non-diabetic and STZ-induced diabetic rats after loading with disaccharides (maltose and sucrose) and the polysaccharide, starch after 18-h fast with and without co-administration of OA. The inhibitory hydrolysis effects of OA against α-amylase, sucrase and α-glucosidase were also investigated *in vivo* and *in-vitro*. Rats administered with deionized water or acarbose acted as untreated and treated positive controls, respectively. By comparison with animals pre-loaded with carbohydrates alone, co-administration of OA with maltose, sucrose and starch significantly reduced the peak blood glucose spikes of separate groups of non-diabetic and STZ-induced diabetic rats. The standard drug, acarbose similarly suppressed the postprandial glucose spikes. The suppression of the postprandial glucose spikes response by OA to carbohydrate loads was associated with the reduction of the area under the blood glucose-time curve ($AUC_{0.5-1\ h}$) of non-diabetic and diabetic animals. By comparison with untreated animals, OA significantly reduced the $AUC_{0.5-1\ h}$ of STZ-induced diabetic rats after loading with maltose (48.61 ± 1.42 vs 36.87 ± 0.91 mmol/l), sucrose (45.87 ± 1.37vs 36.38 ± 0.86 mmol/l) and starch (52.81 ± 1.56 vs 40.95 ± 1.33 mmol/l). The *in vitro* half-maximal inhibitory concentrations (IC_{50}) of OA on α-amylase, sucrase and α-glucosidase were 56.45 ± 1.78, 59.88 ± 1.35 and 62.11 ± 1.79 µg/ml respectively. These results suggest that OA inhibits carbohydrate-hydrolyzing enzymes leading to suppression of postprandial hyperglycaemia in STZ-induced diabetic rats loaded with maltose, sucrose and starch.
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P201

Unfolded protein response pathway, IRE1α-XBP1 is altered during adipogenesis in obese human adipocytes

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Background
Adipose tissue (AT) plays a central role in obesity-related complications such as cardiovascular diseases, insulin resistance, and type two diabetes. A comprehensive understanding of the molecular mechanisms underlying adipocyte formation is of both fundamental and clinical relevance. It has been reported that UPR pathway, IRE1α-XBP1 regulates hepatic lipogenesis and the role of this pathway in adipogenesis in murine adipocytes has also been confirmed. Our team has also shown that this pathway is upregulated in obese human AT. Therefore, we further investigated the role of IRE1α-XBP1 pathway in the process of adipogenesis in lean and obese human preadipocytes.
Methods
A human preadipocyte cell line Chub-S7 ($n=3$) and abdominal subcutaneous (AbdSc) preadipocytes (Ad) from lean ($n=3$, BMI: 22.47($\pm$S.D. 2.55) kg/m^2, age: 35.3($\pm$S.D. 8.50) years) and obese ($n=3$, BMI: 31.97 ($\pm$S.D. 1.26) kg/m^2, age: 31.0 ($\pm$S.D. 7.55) years) individuals were differentiated over 14 days and markers of adipogenesis and IRE1α, XBP1 and spliced XBP1 (XBP1s) were assessed using qRT-PCR and western blotting.
Results
In Chub-S7 adipocytes IRE1α mRNA expression oscillated through differentiation peaking at days 4 and 12, with an eight- and sixfold increased expression relative to day 0, respectively ($P<0.01$, $P<0.01$). A similar pattern of mRNA expression of IRE1α during differentiation of all AbdSc Ad was observed. XBP1s mRNA expression in Chub-S7 cells peaked on day 4 only (90-fold expression relative to day 0; $P<0.01$). XBP1s mRNA expression in obese AbdSc Ad matched that of IRE1α with a 12-fold peak rise on days 4 and 12, whilst in AbdSc Ad from lean subjects XBP1s remained unchanged.
Conclusions
i) The IRE1α-XBP1 pathway is induced during adipogenesis in an oscillatory manner and may have an important role in this process. ii) IRE1α and XBP1s expression was enhanced during differentiation of obese AbdSc Ad compared to lean. These data highlight with weight gain subtle changes in UPR pathway, IRE1α-XBP1 may lead to downstream metabolic consequences.
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P202

Vitamin D and insulin resistance: no association in healthy overweight people at high risk of cardiovascular disease

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Observational studies suggest reduced vitamin D levels are associated with an increased incidence of type 2 diabetes mellitus. We examined the relationship with insulin resistance (assessed using a two-step euglycaemic hyperinsulinaemic clamp technique) in 92 overweight, non-diabetic individuals with no history of cardiovascular disease - mean age 56 years (range 40–77 years), 64% males, 36% females, BMI 30.9 kg/m^2 (range 26.4–36.9 kg/m^2), fasting plasma glucose 5.8 mmol/l (range 4.9–7.0 mg/dl).
Vitamin D was measured using an ultra performance liquid chromatography technique (UPLC) with tandem mass spectrometry. Statistical analysis was performed using Pearson's correlation coefficients and partial correlation.
Geometric mean total vitamin D for the whole group was 32.2 pg/ml. Thirty-three per-cent were deficient (<25 pg/ml), 47% insufficient (26–50 pg/ml), 20% adequate (>50 pg/ml). Geometric mean assessments of insulin resistance were Step one GIR 6.64 μmol/kg per min, Step two GIR 34.70 μmol/kg per min and HOMA-IR 1.83. Pearson's correlation coefficients for vitamin D and GIR step one are −0.003 ($P=0.98$), GIR step two −0.036 ($P=0.73$) and HOMA-IR −0.163 ($P=0.13$). Partial correlation analysis did not detect any significant correlations after correction for potential anthropometric, seasonal or gender confounders. Further subgroup analysis of the deficient group did not detect any significant correlations.
Previous studies have assessed insulin resistance using HOMA-IR and have produced inconsistent results. Using gold standard techniques we did not detect any relationship between vitamin D and measures of whole-body, peripheral or hepatic insulin resistance in this group of overweight healthy individuals at high risk of cardiovascular disease.
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P203

Evaluation of efficacy of transdermal delivery of chloroquine on *Plasmodium berghei*-infected male Sprague–Dawley rats and effects on blood glucose and renal electrolyte handling

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Chloroquine (CHQ) the most frequently used drug for *falciparum* malaria in sub-Saharan Africa countries evokes adverse effects on glucose homeostasis and kidney function in African children. The complications can partly be ascribed to transiently high plasma CHQ concentrations following oral administration and/or malaria parasites. We have, however, reported that topical application of the pectin CHQ matrix patch releases CHQ into the bloodstream. Accordingly, the current study determined whether CHQ delivered via the transdermal route can reduce malaria parasites and ameliorate the side effects associated with oral CHQ. The method of CHQ patch production is similar to that previously reported. Oral glucose tolerance responses (OGT) to CHQ delivered orally or transdermally were monitored in groups of non-infected and *Plasmodium berghei*-infected male Sprague–Dawley rats following glucose load. Blood glucose concentrations were measured at 15-min intervals for the first hour and hourly thereafter for 3 h. Parasitaemia, plasma insulin, blood glucose and renal function were monitored over a 21-day period divided into pre-treatment (days 0–7), treatment (days 8–12) and post-treatment (days 13–21) in separate groups following a once off application of the CHQ patch (53 mg/kg) twice daily administration of CHQ (60 mg/kg, p.o.) during the treatment period. Transdermally delivered CHQ sustained plasma concentrations of CHQ and equally reduced *P. berghei* parasites by comparison with twice daily oral chloroquine. Compared with respective control groups, OGT responses of animals administered oral and transdermal CHQ were lower at all the time points that blood was sampled after the glucose load. Oral CHQ administration increased plasma insulin concentration whilst topical CHQ patch did not have any significant effect. Oral CHQ treatment was associated with increased urinary Na^+ outputs and hyperkalaemia. The CHQ matrix patch did not influence these parameters. We conclude that the CHQ patch has the potential circumvent the adverse effects of oral CHQ.
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P204

Altered mitochondrial dynamics in obesity: redressing the balance through bariatric surgery?

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Mitochondria are essential for synthesising ATP required for cellular metabolism. Mitochondria are able to alter their morphology and abundance through the balance of fission and fusion genes. T2DM is often accompanied by a metabolic syndrome within the individual. The aim of my PhD is to investigate whether metabolic dysregulation in T2DM can be linked to dysfunction within their mitochondria.
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P205

Irisin as a central regulator in energy homeostasis?

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Background/objectives
Irisin is a novel myokine, released predominantly by skeletal muscle. Peripheral action of irisin improves glucose homeostasis, increases energy expenditure and induces browning of adipocytes, with therapeutic potential for use in weight loss. However, there are no human studies on the central role of irisin in the regulation of appetite or energy expenditure. This study sought to examine the potential central role of irisin, by demonstrating irisin in human cerebrospinal fluid (CSF) and hypothalamic sections. We also studied how different metabolic states may alter the effect of irisin, and their relationship with maternal and neonatal serum irisin levels.
Methods
Patients attending for elective Caesarean sections in a tertiary care teaching hospital were recruited. CSF, serum and neonatal cord blood were collected from 91 pregnant women; non-obese ($n=34$) obese ($n=39$) and those with gestational diabetes mellitus (GDM) ($n=18$). Irisin was assessed by an enzyme-linked immunosorbent assay which was validated in-house, using recombinant human irisin.
Results
Irisin was present in human CSF 32.0 (mean $\pm$ S.E.M.) 1.5 ng/ml at a 20–30-fold lower concentration compared with serum irisin (799 $\pm$ 34.5 ng/ml). Serum irisin did not differ between groups but CSF irisin correlated positively with serum irisin levels from non-obese and obese pregnant women ($P<0.01$), with CSF irisin significantly raised in GDM subjects ($P<0.05$). Independent of BMI, serum irisin correlated with HOMA IR ($P<0.01$), cholesterol, TG and HDL ($P<0.01$). Neonatal cord irisin levels (237 $\pm$ 8.1 ng/ml) correlated with maternal serum irisin ($r=0.29$, $P<0.05$). Immunohistochemistry staining of human hypothalamic tissue showed the presence of irisin in the neuronal cells of the paraventricular nucleus, co-localised with neuropeptide Y.
Conclusion
These data suggest a new central role of irisin to potentially influence appetite as well as peripheral energy expenditure which may become desensitised in metabolic disease.

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P206

Ileal interposition with diverted sleeve gastrectomy for treatment of type 2 diabetes

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Objective
To prospectively evaluate the results of laparoscopic ileal interposition (II) with diverted sleeve gastrectomy (DSG) for control of type 2 diabetes mellitus (T2DM) and related metabolic abnormalities.
Methods
All patients underwent II + DSG. They had T2DM $\geq$5 years with poor glycemic control despite adequate dosage of oral hypoglycemic agents (OHAs) and/ or insulin. The primary outcome was remission of diabetes (HbA1C $<$6.5% without OHAs/insulin). Secondary outcomes were reduction in antidiabetic agent requirement and components of metabolic syndrome.
Results
We report the postoperative follow-up data of 13.1 $\pm$ 5.3 months (range:3–26 months). There were 32 patients (Male: female = 21:11) with mean age of 48.7 $\pm$ 7.8 (range, 34–66 years), duration of diabetes of 13.1 $\pm$ 5.8 years (range, 5–30 years), and preoperative body mass index of 29.1 $\pm$ 6.9 kg/ m^2 (range: 22.4–39.5 kg/m^2). They had poorly controlled diabetes with mean FBS: 236.52 $\pm$ 88.4 mg/dl, PLBS: 305. 1 $\pm$ 124.3 mg/dl and HbA1C: 9.8 $\pm$ 1.8%. Sixteen patients (50%) had hypertension, while dyslipidemia and microalbuminuria was present in 12 patients (39%) each.
The mean operative time was 387.7 $\pm$ 84.3 minutes and the mean postoperative hospital stay was 8.8 $\pm$ 5.4 days. Intraoperative complications were noted in four patients (12.5%). Nausea and loss of appetite was observed in three patients (10%), which improved over a period of 2 weeks. At 3 months postoperative follow up, none of these patients had any complications with regards to the intraoperative and immediate postoperative events
Twenty two patients (70.5%) had diabetes remission. Fifteen/ sixteen (93%) patients had remission in hypertension. All participants had weight loss ranging between 15% and 25%. Postoperatively statistically significant decline was observed in the glycemic and lipid parameters, microalbuminuria at all intervals ($P<0.05$). Patients with duration of follow up more than 6 months demonstrated to have better improvement in terms of reduction in glycemic, lipid parameters and microalbuminuria. Three patients had vitamin B12 deficiency 1 year after surgery.
Discussion
The surgery addresses the foregut and hindgut mechanisms for DM control. The DSG component restricts calorie intake and induces ghrelin (orexin) loss. It also excludes the duodenal loop, thereby negating the effect of insulin resistance promoting Rubino's factor. II leads to earlier and rapid stimulation of interposed ileal segment by ingested food resulting in augmented GLP-1 secretion.
Conclusion
II + DSG seem to be promising procedures for control of type two DM and associated metabolic abnormalities.

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P207

Polycystic ovary syndrome has no independent effect on vascular, inflammatory or thrombotic markers when matched for obesity

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Introduction
Previous studies investigating cardiovascular (CV) risk in obese women with polycystic ovary syndrome (PCOS) have been potentially confounded by not adequately accounting for body weight.
Objective
To assess if PCOS increases CV risk independently in young obese women by examining carotid intima-media wall thickness (cIMT) and platelet function.
Design
A cross-sectional study comparing women with PCOS ($n=21$) to age (32.8 $\pm$ 7.2 vs 33.5 $\pm$ 6.7 years), and weight (100.9 $\pm$ 16.7 vs 99.3 $\pm$ 14.7 kg) matched controls ($n=19$). Platelet function was examined by flow cytometry, clot structure and fibrinolysis by turbidimetric assays and endothelial function by ELISA and post-ischaemic reactive hyperemia.
Results
The PCOS group had higher testosterone 1.2 $\pm$ 0.3 vs 0.9 $\pm$ 0.3 nmol/l ($P=0.01$), HOMA-IR 2.5 $\pm$ 1.7 vs 1.7 $\pm$ 1.0 ($P=0.08$), impaired glucose tolerance 33.3% vs 5.3% ($P=0.02$), and urinary isoprostane 16.0 $\pm$ 4.4 vs 11.8 $\pm$ 7.1 ng/ml ($P=0.04$) compared to controls. Mean cIMT 0.5 $\pm$ 0.05 vs 0.48 $\pm$ 0.06 mm ($P=0.36$), and basal platelet surface expression (percentage of positive cells) of P-selectin 0.52 $\pm$ 0.3 vs 0.43 $\pm$ 0.23 ($P=0.40$) and fibrinogen binding 0.97 $\pm$ 0.4 vs 0.83 $\pm$ 0.3 ($P=0.48$) did not significantly differ between the PCOS and control groups, respectively. Furthermore, platelets sensitivity to stimulation with adenosine-five'-diphosphate or inhibition with prostacyclin, clot structure and fibrinolytic efficiency *ex vivo*, endothelial reactive hyperemic index (RHI), inflammation (hsCRP) and adhesion markers (sE-selectin, sP-selectin, sVCAM-1 and sICAM-1) were not significantly different between the two groups.
Conclusions
PCOS appeared not to independently increase atherothrombotic risk when matched for obesity. It is likely that any excess CV risk in young obese women with PCOS can either be attributed to obesity or is not yet apparent at this early stage of the condition.

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P208

Leptin in serum and vitreous humor, and retinal *ob* mRNA, in human diabetes

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Introduction
Elevated intraocular (vitreous) leptin is implicated in proliferative diabetic retinopathy. The source of vitreous leptin is uncertain and it could be derived from systemic leptin. However we showed that leptin (*ob*) mRNA is present in rat retina, suggesting that retinal tissue could also be a source of intraocular leptin. The present investigation quantified *ob* mRNA in human retinal tissue obtained from diabetic and control cadavers. In a parallel study, non-diabetic and diabetic patients provided serum and vitreous samples for determination of leptin concentrations.
Methods
Human retinal tissue (CON, $n=8$; DIAB, $n=8$) was obtained from the Eye Bank of Canada (Toronto). *Ob* mRNA was quantified by real-time RT-PCR. Serum and vitreous samples were obtained from diabetic ($n=35$) and non-diabetic ($n=25$) patients. Leptin concentrations were determined by radioimmunoassay (Linco Research, Missouri; sensitivity, 0.05 ng/ml).
Results
Serum leptin was not significantly different in DIAB vs CON groups. However differences were seen when male (M) and female (F) groups were compared: F DIAB had higher serum leptin than both CON and M DIAB ($P<0.001$), whereas M DIAB vs CON values were not different. In contrast, vitreous levels of leptin were increased in all diabetics ($P<0.01$) and vitreous leptin levels were also affected by sex; eg. F DIAB values >M DIAB ($P<0.001$). *Ob* mRNA was present in all retinal samples. Levels were higher in DIAB vs CON (+60%; $P=0.01$) and a sex difference was also seen (F>M; $P<0.01$).
Conclusions
We observed a significant positive effect of diabetes on vitreous leptin levels, especially in females. Since serum leptin was also higher in females compared to males, it suggests that elevated systemic leptin concentrations could be responsible, at least in part, for the high levels observed in vitreous samples from diabetic patients. Nonetheless, we confirmed that human retina might be a source of intraocular leptin, and *ob* mRNA levels were increased by diabetes in females.
Declaration of funding
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P209

The endocrine and metabolic characteristics of a large Bardet–Biedl syndrome clinic population

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Background
Bardet Biedl syndrome (BBS) is a rare autosomal recessive disorder caused by ciliary dysfunction. It is characterised by rod cone dystrophy, polydactyly, renal dysfunction, cognitive impairment. Endocrine consequences are thought to include hypogonadism, obesity and polyuria. However little is known about the endocrine and metabolic abnormalities in adult patients.
Methods
One hundred and fifty-four patients with BBS were identified through two national BBS clinics; anthropometric measurements and fasting blood samples were taken in 130 patients. Date are reported as mean ± S.E.M.
Results
Eighty-five patients (55.2%) were male. The age was 33.0 ± 1.0 years (range 13–58 years) and BMI was 35.7 ± 0.7; 102 (76.7%) were obese (BMI >30 kg/m^2). The prevalence of metabolic syndrome was high (59.4%) and 24(15.6%) BBS patients had type 2 diabetes; one patient had type 1 diabetes. Polycystic ovary syndrome was present in 12/69 (17.4%) females and 35 (27.3%) patients had biochemical evidence of NAFLD.
Most BBS patients were euthyroid (77.2%), 10(6.5%) had hypothyroidism and one had hyperthyroidism. Twenty-four (19%) had subclinical hypothyroidism.
One hundred and two BBS patients (78.4%) were eupituitary. Of the remaining patients, 15 (11.5%) had an isolated low IGF1, five had mild hyperprolactinaemia (prolactin <1000 mIU/l) and seven patients had isolated low prolactin. One patient had significant hyperprolactinemia (prolactin=6391 IU/ml) and subsequent MRI showed pituitary hypoplasia.
Twenty-six (40%) males were hypogonadal (primary in four patients and secondary in 22 patients). Two patients had nephrogenic diabetes insipidus.
Four (3.9%) patients had stage 5 CKD, 4(3.9%) stage 4 CKD and 14 (13.7%) stage 3 CKD. Four patients had functioning renal transplants.
Conclusions
This is the first study to investigate endocrinopathies in a large BBS population. Despite previous reports, generalised pituitary hormone dysfunction is not prevalent but subclinical hypothyroidism and hypogonadism are common. The majority of patients are obese and the prevalence of metabolic syndrome and type 2 diabetes is high.
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P210

Maternal B12 insufficiency predicts neonate's metabolic risk factors

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Studies in India (vegetarian population) show that vitamin B12 insufficiency is common in pregnancy and independently predict adiposity and insulin resistance in the offspring. Epigenetic programming is postulated as, along with folic acid, B12 is crucial for DNA methylation. Therefore, we investigated whether maternal B12 levels in a non-vegetarian UK population predict metabolic risk of the offspring.
Paired maternal venous and cord blood samples ($n=91$) were collected at the time of elective caesarean section. Serum vitamin-B12 and folate were determined by electro-chemiluminescent immunoassay. Serum homocysteine was determined by liquid-chromatography with tandem-mass spectrometric detection (LC–MS/MS).
B12 insufficiency (<150 pmol/l) was common (mothers 40%; neonates 29%) but not folate insufficiency (<7 nmol/l) (11% only in mothers). Low B12 mothers had significantly higher BMI, triglycerides, cholesterol, LDL-cholesterol and homocysteine. The neonates of low B12 mothers had higher triglycerides, leptin, homocysteine and lower HDL-cholesterol. Correlation analysis showed that maternal B12 ($r=0.648$; $P<0.001$), folate ($r=0.706$; $P<0.001$) and homocysteine ($r=0.756$; $P<0.001$) are associated with the respective neonatal indices. Furthermore, maternal B12 was inversely associated with neonatal triglycerides, HOMA-IR, leptin, homocysteine and directly with HDL-cholesterol. In multiple regression analysis, adjusted for all likely confounders, maternal B12 independently predicted neonatal HDL-cholesterol ($\beta=0.169$; $P=0.044$; $R^2=8.6\%$), leptin ($\beta=-0.662$; $P=0.002$; $R^2=12.7\%$), homocysteine ($\beta=-0.302$; $P=0.001$; $R^2=14.7\%$) but not triglycerides or HOMA-IR.
Our study replicates the Indian observations in a non-vegetarian population that maternal B12 insufficiency predicts metabolic risk of the offspring, albeit in a selective group at delivery. Carefully designed studies with appropriate control group, looking at early pregnancy B12 are warranted to assess the causality of our observation. If replicated, this has the potential to reduce the burden of metabolic disorders in the offspring.
Declaration of funding
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P211

The use of GLP-1 receptor agonists to manage unwanted weight gain in patients with 'hypothalamic' obesity secondary to structural pituitary pathology

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Introduction:
The hypothalamus is intimately involved in weight homeostasis. Pituitary tumours and treatment for pituitary tumours has been well described to induce obesity in certain subjects presumably due hypothalamic irritation or dysfunction. This is often very challenging to treat.

GLP-1 analogues have direct central effects and have been shown to be effective for weight loss in obese patients with and without diabetes mellitus.
Aims
To perform a pilot study to look at the effectiveness of GLP-1 treatment in patients with hypothalamic obesity secondary to pituitary pathology.
Method
We treated six patients and looked at weight loss, tolerability and safety.
Results
six patients received GLP-1 analogue. three had pre-existing type 2 diabetes mellitus. Mean age of the subjects was 49 ± 8.8 years. Mean BMI was 45.3 ± 11.7 kg/m^2. All patients but one had pituitary surgery for various pituitary tumours. All patients had a large increase in body weight following the development or treatment of pituitary pathology four patients had liraglutide and two had exanatide. Duration of treatment ranges from 2 months to 47 months. Net weight loss ranges from −4.7 to −16.6 kg. An average weight loss was 8.5 kg per subject over a variable duration of treatment. All subjects tolerated GLP-1 analogue well. In those subjects who had diabetes mellitus, all subjects were well controlled prior to GLP-1 intiation (HbA1c 53 mmol/mol) and this did not change
Discussion
To our knowledge, the use of GLP-1 analogue in patients with pituitary pathology and hypothalamic obesity has not been reported before. Our experience showed that GLP-1 analogues are not only effective but also clinically safe in managing obesity in pituitary patients with or without diabetes mellitus. Further research into the use of GLP-1 analogue in pituitary patients may be useful.

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P212

The public distress domain of quality of life correlates directly and independently with BMI in pre-operative morbidly obese patients awaiting metabolic surgery

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Background
There is uncertainty regarding the selection of obese patients for metabolic procedures, how to define a successful outcome and pre-operative predictors of success. Our aim was to explore the influence of obesity on preoperative quality of life in patients awaiting bariatric surgery.
Methods
Pre-operative data were accrued for morbidly obese patients ($n=70$) at the University Hospitals Coventry and Warwickshire (UHCW) during the last 2 years, and for whom funding for metabolic surgery had been secured. Baseline pre-operative assessment details including clinico-demographic data and where available, IWQOL-Lite questionnaire (a validated self-reported 31-item measure of physical function, self-esteem, sexual life, public distress and work related domains of obesity-specific quality Of life (QOL)) scores transformed to a 0 to 100 scale, where a score of 100 represents the best Health-Related QOL (HRQOL), were obtained. The impact of BMI on each QOL measure was analysed through bivariate Pearson correlations and through comparisons between two sub-groups based on body mass index (BMI): 40-49.9 kg/m^2 and ≥60 kg/m^2. Results are reported as (mean) and (S.D.). $P<0.05$ is considered statistically significant.
Results
The lower BMI ($n=30$) and higher BMI ($n=12$) sub-groups had no significant differences in age and sex distribution. Comparisons between the lower and higher BMI sub-groups showed significant difference in public distress HRQOL alone (34.6 (27.2) vs 14.5 (21.8) respectively; $P=0.03$). Bivariate analyses showed significant negative linear correlation only between BMI and public distress HRQOL ($r=-0.341$, $P=0.002$). On multivariate linear regression analysis with BMI, age and sex as independent variables and HRQOL scores as dependent variables, the impact of BMI on public distress was significant ($B=-1.339$, $P=0.002$).
Conclusion
BMI appears to influence all domains of QOL in a negative linear fashion but is only significant for public distress HRQOL. Improvements of HRQOL measures should feature in the definition of a successful metabolic surgical outcome.

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P213

Pregestational BMI predicts neonatal hypoglycemia in women with gestational diabetes

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Background and aims
In diabetic pregnancy, neonatal hypoglycemia (NH) is usually attributed to insufficient regulation of maternal glycemic control. Recent data suggest that maternal BMI could have an influence on NH. The objective of this study was to determine whether an association exists between maternal prepregnancy BMI and occurrence of NH among infants born to women with gestational diabetes mellitus (GDM).
Materials and methods
This was a retrospective study including all GDM pregnancies delivered between 2007 and 2011 at a tertiary care center hospital. GDM was diagnosed using the ADA criteria. Three hundred and sixty-two newborns were studied. NH was defined according to Cornblath criteria. In addition to maternal BMI (according to Asian-Indian criteria), other variables such as glycemic status at diagnosis or third-trimester glycosylated hemoglobin as potential predictors of NH were also studied. We also explored whether the association between maternal BMI and NH could be due to factors such as caesarean section or abnormal birth weight.
Results:
The rate of NH was 2.9%. In the bivariate analysis, prepregnancy BMI was higher in the NH group (27.15 vs 24.07 kg/m^2, $P<0.02$). In the logistic regression analysis, prepregnancy BMI of at least 26.7 kg/m^2 was independently associated with NH whether the analysis included other factors (odds ratio = 2.21; 95% CI = 1.29–3.45) or not (odds ratio = 2.85; 95% CI = 1.66–5.76).
Conclusions
Pregestational BMI should be considered among the predictors of NH in offspring of women with GDM.

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P214

Effectiveness of bariatric surgery in women with and without polycystic ovarian syndrome

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Introduction
The prevalence of clinical obesity in women with polycystic ovarian syndrome (PCOS) is 50%. Weight loss is effective at enhancing insulin sensitivity, reducing hyperandrogenaemia, improving hirsutism and restoring menstrual regularity and fertility in PCOS. However, women with PCOS appear to be less responsive to weight loss interventions including some bariatric surgical procedures. The aim of this study was to compare weight loss outcomes of gastric bypass surgery in women with and without PCOS.
Methods
We performed a retrospective, comparative cohort analysis of weight loss, blood pressure (BP) and HbA1c following gastric bypass surgery in women with and

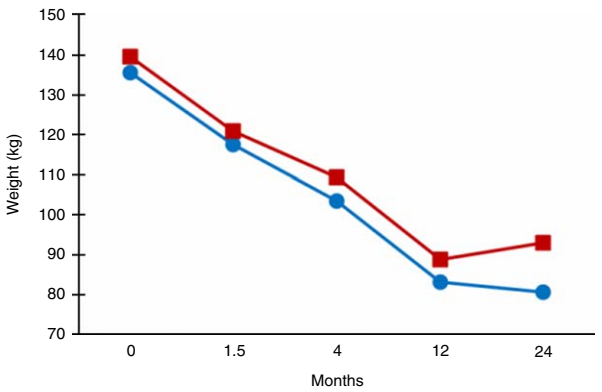

Figure 1 Weight loss following gastric bypass surgery in women with PCOS (blue circles) and without PCOS (red squares).

without PCOS aged 18–50 years matched for age (±5 years) and preoperative BMI (±5 kg/m^2). We report results from 56 women, 28 with and 28 without PCOS.
Results
Mean preoperative age was 28.5 years, weight 137.5 kg, BMI 50.0 kg/m^2, systolic and diastolic BP 135 and 85 mm Hg respectively, and HbA1c 37 mmol/mol; there were no significant differences between the two groups. There was significant weight loss following bariatric surgery with no statistical difference between groups (see Fig. 1). Likewise, there was significant reduction in BMI, BP and HbA1c with no significant difference between groups.
Conclusion
Gastric bypass surgery in obese women with PCOS results in significant reductions in weight, BMI, BP and HbA1c, similar to women without PCOS.

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P215

Octreotide therapy of chronic urticaria and angioedema after gastric bypass procedure

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Background
The exact changes of hormones and the relative importance of these to the metabolic improvement after bariatric surgery remain to be explored. We highlight the unusual case of a patient who developed episodes of angioedema post roux-en-y surgery for weight reduction.
Case
A 38-year-old man with a BMI of 50 kg/m^2, had laparoscopic Roux-en-y bypass surgery, resulting in 89 kg loss over 12–18 months. Six to twelve 12 months post-op he developed recurrent facial angioedema and mild urticaria, lasting for 6–12 h, relieved with oral steroids and antihistamines. Two of these episodes were associated after taking ibuprofen. A low salicylate diet was ineffective as were multiple antihistamines at up to triple standard doses at preventing the reactions. Tests for autoimmunity, food allergy, C1 inhibitor deficiency, mast cell tryptase, pheochromocytoma, and carcinoid including octreotide scintigraphy were negative. His plasma gut peptide profile was within the normal range. As several urinary 5-HIAA levels were borderline elevated (52.8, 54.1 and 36.5 μmol/day, normal 0–50) he was commenced on octreotide and has since remained symptom free. Attempts to reduce the octretide dose result in return of symptoms, indicating that he does not just have chronic urticaria that has now resolved.
Discussion
There is progressive rise in peptideYY, enteroglucagon, pancreatic polypeptide and GLP-1 after gastric bypass surgery. Somatostatin and its analogue octreotide inhibit the release of peptide hormones through stimulation of somatostain receptors and inhibition of L-type calcium channels. Octreotide induced modulation of post bypass satiety gut hormone release is proven in animal models. We hypothesise the response seen in this case is due to modulation of certain gut peptide(s) with angioneurotic properties.
Conclusion
Trials of octreotide therapy may be useful in similar patients.

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P216

Prevalence of vitamin D insufficiency in severely obese patients seeking bariatric surgery

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Background and objectives
Vitamin D deficiency and insufficiency is common in obese individuals. We aimed to determine the prevalence of vitamin D insufficiency within a cohort of severely obese individuals, exploring potential underlying associations.
Methods
In a retrospective analysis of 703 patients sequentially presenting for bariatric surgery assessment, 663 had complete demographic, anthropometric, and haematological measures (vitamin D, folate, vitamin B_1, vitamin B_{12}, white cell count, and C-reactive protein). Vitamin D levels were defined as deficient (< 25 nmol/l), insufficient (25–50 nmol/l), or normal (> 50 nmol/l). Adiposity was estimated using indirect measures; BMI, and body fat percentage (BF%) estimated via the CUN-BAE equation. Statistical analyses: groups formed according to vitamin D status were compared using ANOVA. Stepwise regression was performed using age, gender, BMI, BF%, index of multiple deprivation, multivitamin use, number of comorbidities, white cell count, C-reactive protein, folate and vitamin B_{12} as independent variables for vitamin D concentration.
Results
Mean serum vitamin D concentration was 38±23 nmol/l. 75% of patients had abnormal serum vitamin D concentrations; 33.2% were deficient, and 41.8% insufficient. Individuals were divided according to vitamin D status (deficient vs insufficient vs normal). Those with vitamin D deficiency had an increased BMI (48.6 vs 46.9 vs 45.4 kg/m^2; $P<0.001$), decreased folate concentrations (5.8 vs 7.7 vs 8.7 ng/ml; $P<0.001$), and decreased vitamin B_{12} concentrations (393 vs 445 vs 464 pg/ml; $P=0.003$). Stepwise regression revealed only a weak association with folate concentration ($R^2=0.081$; $P=0.046$).
Conclusion
Vitamin D insufficiency is common among severely obese bariatric surgery candidates, observed in approximately 75% of patients. The underlying cause is still unknown and the hypotheses of sequestration within adipose tissue, or inadequate nutritional intake are not supported by these data. Questions remain regarding the functional significance of low vitamin D concentrations among obese individuals, and whether routine supplementation is required.

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P217

Appetite regulation during a 6-month military tour to Afghanistan

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Background
Military personnel on operational deployment commonly lose weight despite the adequate supply of rations. Moderate weight loss (~5% body mass) occurred during the initial phase of a 6-month deployment to Afghanistan without affecting physical fitness. Reasons for this weight loss are presently unknown. We sought to establish whether changes in appetite regulatory hormones contribute to the observed weight loss.
Methods
Body mass and body composition were measured in a cohort of Royal Marines twice before (January and March) and during a 6-month summer (March–October) deployment to Afghanistan (Afgh) in 2010. Circulating total and active ghrelin, peptide YY, pancreatic polypeptide, glucagon-like peptide-one, insulin and leptin were measured in samples drawn at the same time points. Data were analysed by repeated measures ANOVA.
Results
Percentage body fat increased between the January and March time-points taken in the UK, but was reduced after (mean) 3 months in Afgh (17.5 ±0.5% (Jan) vs 18.0 ±0.5% (Mar) vs 16.5 ±0.5% (Afgh); $n=105$; $P<0.0001$); changes in serum leptin concentrations in this lean population mirrored those observed in percentage body fat (2.15 ±0.20 ng/ml (Jan) vs 2.76 ±0.20 ng/ml (Mar) vs 1.44 ±0.12 ng/ml (Afgh); $n=51$; $P<0.0001$). Only leptin was significantly correlated with percentage body fat (and body mass) at each time-point ($r=0.59$; $n=122$ (Jan), $r=0.58$, $n=137$ (Mar), $r=0.51$, $n=98$ (Afgh); all $P<0.0001$). There were no changes in the concentrations of the other hormones measured in response to alterations in body mass or body fat.
Discussion
Leptin has previously been shown to be reduced after weight loss in obese populations also after short-term (72 h) starvation in lean volunteers. The current study suggests that fasting leptin concentration also reflects small changes in percentage body fat in a lean population of military personnel. Future work will investigate whether post-prandial changes in gut hormones may drive the alterations in energy homeostasis observed on deployment.
Declaration of funding
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P218

Weight loss after bariatric surgery in women of childbearing age

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Obesity increases the risk of pregnancy-related complications such as miscarriage, foetal abnormalities, hypertension, diabetes, thrombosis, caesarean section and infection. Although bariatric surgery addresses some of these risks, women should defer pregnancy for 12-24 months postoperatively until weight loss has plateaued due to concerns regarding limited maternal weight loss and foetal nutritional deficiency. The aim of this study was to evaluate the impact of pregnancy on weight loss after bariatric surgery and to assess pregnancy outcomes.

Amongst 730 obese people who underwent bariatric surgery, 232 women of childbearing age (18–45 years) with a mean ± S.D. age 34.0±5.9 years, preoperative weight 137.7±21.3 kg and BMI 50.6±7.2 kg/m^2 were identified. One-hundred and ninety-seven women (84.9%) had undergone Roux-en-Y gastric bypass, 19 (8.2%) adjustable gastric banding, 8 (3.4%) sleeve gastrectomy and 8 (3.4%) other procedures.

Women who became pregnant following bariatric surgery ($n=21$) were younger at the time of surgery compared to women in the non-pregnancy group (28.0±5.4 vs 34.6±5.6 years, $P<0.001$) but were otherwise well matched for preoperative weight (136.5±18.5 vs 137.8±21.6 kg), BMI (49.2±7.4 vs 50.7±7.2 kg/m^2) and type of bariatric procedure.

Eighteen women (86%) completed pregnancy successfully; 12 (57%) had live births by vaginal route, 6 (29%) had caesarean section; 2 (9%) undertook medical termination of pregnancy and 1 (5%) suffered a spontaneous miscarriage.

At a median follow-up of 30 months, the pregnancy and non-pregnancy groups achieved significant but comparable excess weight loss after bariatric surgery (70.4 vs 70.0%).

In conclusion, pregnancy after bariatric surgery is safe and does not adversely influence weight loss outcomes. However, close surveillance of maternal weight and nutritional status is advisable, especially with conception within the first 12 months after surgery.

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P219

Eating more quickly heightens overall systemic exposure to glucose and NEFA in the post-prandial phase, irrespective of energy expenditure in obese women

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Background/aim

The global obesity epidemic has promoted a search for novel solutions. One approach is through modification of eating-related behaviours. Our aim was to explore the effects of meal duration on energy expenditure, appetite and excursions of molecules associated with insulin sensitivity in the post-prandial phase.

Methods

Normoglycaemic, pre-menopausal, Caucasian obese women ($n=8$) were recruited from the Obesity clinic at Warwickshire Institute for the Study of Diabetes, Endocrinology and Metabolism (WISDEM, UHCW). Whole-body indirect calorimetry (6-h) was performed on each subject on two separate occasions, with standard lunch (763 kcal: 50% carbohydrate) ingested over 10- vs 40-min. Blood tests were taken at baseline (fasting) and at 30, 60, 90, 120, 180 and 240 min following mid-meal, and analysed for glucose, insulin and non-esterified fatty acids (NEFA). Number/composition of calories ingested during a standard buffet meal (240-min) was assessed. Paired-sample t-tests were used. $P<0.05$ was considered statistically significant.

Results

Overall, following the 10- vs 40-min meals, there were significant differences in post-prandial plasma glucose (mean 6.4 mmol/l (S.D. 1.4) vs 6.0 mmol/l (S.D. 1.3) respectively, $P=0.01$) and serum NEFA concentrations (mean 243 μmol/l (S.D. 224) vs 191 μmol/l (S.D. 171) respectively, $P=0.005$). There was a trend towards greater post-prandial insulin resistance following the 10- vs 40-min meals (HOMA2 IR 2.1 (S.D. 2.0) vs 1.9 (S.D. 1.7) respectively, $P=$NS). Post-prandial energy expenditures were equivalent between the two meal durations (range 80–120 kcal/h). Regarding buffet selection ($t=240$-min), total number/composition of calories ingested were equivalent between the meal durations.

Conclusion

Our novel data highlight that meal duration impacts on systemic glucose and NEFA in obese women. Eating a meal more quickly increases overall exposure to glucose and NEFA, with a trend towards increased insulin resistance during the post-prandial phase; whilst no impact was noted on energy expenditure or appetite. Longer meal durations may therefore facilitate better maintenance of metabolic health.

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P220

Hepatic 11β-hydroxysteroid dehydrogenase type 1 is elevated following weight loss secondary to bariatric surgery

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In the pathogenesis of obesity, dysregulated tissue cortisol metabolism (controlled by 11β-HSD1), is postulated to be involved. Fifteen patients (seven mens, mean BMI 50.8±7 kg/m^2) awaiting Roux En Y gastric Bypass (RYGB) surgery underwent assessment of 11β-HSD1 activity using cortisol generation profile. Corticosteroids in serum and subcutaneous adipose tissue microdialysis fluid and urinary corticosteroid metabolites were analysed by liquid and gas chromatography mass spectrometry. Results were compared to six *post* op patients and non-obese controls. Mean area under the curve (AUC) for serum cortisol (F) to cortisone (E) ratio (marker of 11β-HSD1 activity) was significantly reduced in pre-op patients compared to *post*-op patients: 1037±448.9 vs 1656 ±505.1 units/min ($P=0.0045$). Subcutaneous adipose tissue Microdialysis fluid showed a threefold reduction in F/E AUC ratio following RYGB surgery: 303.2 vs 103.2 units/min. Total urinary cortisol metabolites (Fm) were significantly raised in severely obese patients compared to non-obese controls; 6602±742 vs 4396±2362 μg/24 h ($P=0.026$). The ratios of (THF +5αTHF)/THE (marker of 11β-HSD1 activity) and Fm/Em were not significantly different. The ratios of androsterone/etiocholanolone and 5′ tetrahydrocortisol/5α tetrahydrocortisol (markers of 5α reductase activity) were not significantly different. In this obese cohort, there is evidence of hypothalamic–pituitary–adrenal axis activation as reflected by increased total cortisol metabolites, driven by decreased hepatic 11β-HSD1 generation of cortisol. Global urine metabolites are not different reflecting possible up regulation of 11β-HSD1 in adipose tissue and muscle, and down regulation in liver. This may reflect a protective mechanism to reduce hepatic cortisol exposure.

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P221

Brown adipose tissue identification in an adult human using IDEAL MRI

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Aim
Manipulation of human brown adipose tissue (BAT) represents a novel therapeutic option for diabesity. The aim of our study was to develop and test a novel magnetic resonance imaging (MRI) based method to identify human BAT and delineate it from white adipose tissue (WAT), and validate it by providing immunohistochemical confirmation.
Methods
Initial scanning with ^{18}F-FDG PET–CT radiotracer uptake on a 25-year-old Caucasian female with primary hyperparathyroidism, showed avid uptake within the mediastinum, neck, supraclavicular fossae and axillae, consistent with BAT. Subsequently, serial MR scans were performed using three-echo IDEAL (iterative decomposition of water and fat with echo asymmetry and least-squares estimation) sequence. Retrospectively, regions of interest (ROIs) were identified on MR corresponding to PET–CT images. Prospectively, ROIs were identified on MR images based on signal intensity and appearance, and compared with PET–CT. Immunohistochemical staining using uncoupling protein-1 antibody was performed on fat samples corresponding to low MR-signal, obtained during parathyroidectomy.
Results
Of the 111 retrospectively identified ROIs from PET–CT scans, 88 (79%) showed corresponding low signal on the MR images: 100% in mediastinum, 29/31 (93.5%) in neck, 31/41 (75.6%) supraclavicular, and 8/14 (57%) in axillae. Prospectively, 87% of ROIs identified on MR scans corresponded to increased areas of uptake on PET–CT scans. Histology and immunohistochemistry confirmed BAT.
Conclusion
We provide the first ever report that MR can be used reliably to identify BAT in a human adult, with histological and immunohistochemical confirmation. Our data demonstrate proof of concept to support the development of MR, a safe and reproducible imaging modality, as a biomarker for human BAT.

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P222

Vitamin D supplementation as influenced by diabetic therapies

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Objective
Vitamin D deficiency is associated with an increased risk of type two diabetes mellitus (T2DM) and cardiovascular disease reduced through Vitamin D3 supplementation. However to the best of our knowledge, no research has determined the effect of vitamin D3 supplementation in conjunction with existing pharmacological and non-pharmacological approaches to the DMT2 population. Hence, the aim of this study was to determine the effect of vitamin D3 supplementation in a cohort of Saudi DMT2 population on different oral hypoglycemic agents and compare them with a non-DMT2 control.
Methods
A total of 499 randomly selected DMT2 subjects divided into eight groups based on their existing diabetes management (non-DMT2 control $=151$; rosiglitazone (Avandia) $=49$; diet $=15$; insulin alone $=55$; insulin in combination with other oral hypoglycemic (OHG) agents $=12$; metformin alone $=121$; OHG combination $=37$; sulphonylurea $=59$) were included in this 12-month interventional study. All subjects were given 2000 IU vitamin D3 daily except the control group. Anthropometrics, glucose, lipid profile and 25-OH Vitamin D were measured at baseline, 6 and 12 months.
Results
T2DM subjects treated with Metformin raised vitamin D2 levels at 6 and 12 months compared with baseline ($P<0.001$). No significant changes were observed between Vitamin D2 levels and BMI, glucose, total- and HDL-cholesterol in any DMT2 groups. However significant decreases in triglycerides were observed in subjects treated with both rosiglitazone and insulin + OHG group at 6 and 12 month Vitamin D3 supplementation (both P values <0.001).
Conclusion
Whilst all groups increased circulating vitamin D2 following supplementation, it appears that DMT2 patients on insulin in combination with other OHG drugs heighten their Vitamin D3 absorption. As such Vitamin D3 taken with insulin plus OHG can offer benefits to reduce Vitamin D deficiency whilst improving cardiometabolic risk factors.
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P223

Association of the incidence of type 1 diabetes with markers of infection and antibiotic susceptibility at country level

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Aim
To investigate the association between country incidence of type one diabetes (T1DM) and mortality from infectious disease as well as to antibiotic susceptibility
Materials and methods
An ecological study correlating data from the WHO DiaMond Project for the incidence of T1DM, the WHO estimates of mortality (2004) from communicable diseases and the Alexander Project for bacterial susceptibility to antimicrobial agents.
Results
There were statistically significant negative correlations between the incidence of T1DM and mortality from: infections and parasitic diseases ($r=-0.34$, $P=0.01$) , respiratory infections ($r=-0.29$, $P=0.03$), tuberculosis ($r=-0.36$, $P=0.007$) , diarrhoeal diseases ($r=-0.32$, $P=0.02$) and total infectious disease mortality ($r=-0.35$, $P=0.008$). There was a positive correlation between T1DM incidence and susceptibility of *Streptococcus pneumoniae* to penicillin ($r=0.47$, $P=0.03$), erythromycin ($r=0.52$, $P=0.014$), doxycycline ($r=0.65$, $P\leq0.002$) and co-trimoxazole ($r=0.58$, $P=0.007$). We also found a positive correlation between T1DM incidence and the mean susceptibility ($r=0.62$, $P=0.004$), and lowest antibiotic susceptibility ($r=0.73$, $P\leq0.0001$) of *S. pneumonia.*
Conclusion
We found a negative correlation between country incidence of T1DM and its mortality from infectious diseases. Mortality from infectious diseases is a strong marker of the total infective burden. Incidence of T1DM was found to be positively correlated with the susceptibility of *S. pneumoniae* to all antibiotics studied. Increased antibiotic susceptibility of a given organism may be an indirect marker of a low degree of exposure of the community to it. Our results provide support for the hygiene hypothesis, namely that diminished bacterial exposure in early post-natal life results in increased risk of developing T1DM.

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P224

A pilot study of 25-hydroxy vitamin D level in type 2 diabetes mellitus with diabetic retinopathy

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Background
Many cellular, preclinical and observational studies support a role for vitamin D (VD) in the pathogenesis of type 2 diabetes. VD is suggested to be an inhibitor of angiogenesis. A growing body of evidence suggests an association between VD inadequacy and diabetic retinopathy.
Objective
To study the relation between 25 (OH) vitamin D level and diabetic retinopathy (DR) in patients with type 2 diabetes mellitus and to evaluate for any relation between 25 (OH) vitamin D level and different stages of diabetic retinopathy.
Subjects and methods
50 Type 2 diabetic patients and 50 healthy volunteers (control group) matched by age and sex participated in the study. Based on their ophthalmic findings, the type 2 diabetic patients were divided into two groups: group (I) 25 patients with DR and group (II) 25 patients without DR. Fluorescein angiography was done for

group (I) and accordingly the patients were further classified into three subgroups: moderate NPDR, severe NPDR and PDR. Each subgroup is then divided according to presence or absence of clinically significant macular edema (CSME). Fasting blood sugar, HbA1c, renal functions, liver functions, lipid profile, serum calcium, serum phosphorous, intact parathyroid hormone (iPTH) and serum 25 hydroxyvitamin D_3levels were done to all participants in the study.
Results
Mean 25(OH) VD level was lower in type 2 diabetic cases than in control group ($P<0.01$). Mean 25(OH) VD level was lower in type 2 diabetic cases with DR than type 2 diabetic cases without DR ($P<0.05$). Patients with PDR have the lowest mean 25(OH) vitamin D level compared to patients with moderate NPDR and severe NPDR ($P<0.05$). 25(OH). VD level was inversely correlated with age, duration of type 2 diabetes mellitus, stages of diabetic nephropathy, fundus findings, BMI, SBP, DBP, glycemic parameters, urinary ACR, total cholesterol, triglycerides, LDL-C ($P<0.01$) and iPTH level ($P<0.05$). 25(OH) VD level was positively correlated with GFR, HDL-C and total calcium level.
Conclusions
There is an association between 25 (OH) vitamin D insufficiency and DR among patients with type 2 diabetes mellitus. Low serum 25 (OH) vitamin D might be a risk marker of development or progression of DR. Measurement of serum 25 (OH) vitamin D concentrations could become a useful biochemical marker related to the severity of DR. The effect of vitamin D replacement in patient with DR should be evaluated.

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P225

Non-alcoholic fatty liver disease in patients attending the National Severe Insulin Resistance Service

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Introduction
The prevalence of non-alcoholic fatty liver disease (NAFLD) is greatly increased in patients with lipodystrophy and some other forms of severe insulin resistance. Liver biopsy remains the definitive technique for diagnosis and staging of NAFLD. However, non-invasive techniques, such as magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI) are increasingly used to assess response to therapeutic interventions. The National Severe Insulin Resistance (SIR) Service, commissioned by the National Specialist Commissioning Team in 2011, aims to optimise outcomes of patients with lipodystrophy and/or SIR. Selected patients, including all those receiving leptin therapy, are offered annual measurement of liver fat using MRS or MRI.
Patient population
Liver fat was measured in 28 patients (23 female), median (range) age 28 (13–58) years, BMI 25.8 (15.8–34.0) kg/m^2. Twenty-four have lipodystrophy and four have SIR of currently unknown cause. None have an insulin receptor mutation. 11 were taking leptin. 13/28 patients were scanned using proton single voxel MRS and 15/28 patients were scanned using in-phase and out of phase gradient-echo MRI with dual flip angles.
Results
23/28 (82.0%) patients had >5% liver fat. Median (range) liver fat was 22.6% (1.4–102.0) in patients scanned by MRS and 9.0% (2.0–29.0) in those scanned by MRI. Biopsies were available in two patients. Interestingly these patients both have biopsy proven fibrotic liver disease, but relatively low liver fat by MRI of 4.0 and 6.0% respectively.
Conclusion
As expected, there is a very high prevalence of NAFLD in patients with lipodystrophy and/or SIR attending the National SIR Service. MRI/S based assessment of hepatic steatosis may be useful in assessing response to therapy, but a normal result does not preclude the presence of significant NAFLD and may even signify the presence of fibrosis in some cases, though this is yet to be formally proven.
Declaration of funding
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P226

A study of serum osteocalcin level in women with gestational diabetes mellitus

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Introduction
Gestational diabetes mellitus complicates up to 14% of pregnancies worldwide. Gestational diabetes mellitus women are more frequently affected by changes in bone turnover during pregnancy. Osteocalcin, ostoblast derived protein is suspected to be involved in the regulation of glucose and fat metabolism.
Aim of the work
is to study serum osteocalcin levels in women with gestational diabetes mellitus.
Subjects and methods
This study included sixty pregnant women at 22nd to 28 weeks of pregnancy. They are subdivided into 30 women diagnosed with gestational diabetes mellitus and 30 healthy pregnant women. All subjects included subjected to full history taking, clinical examination and laboratory investigations which includes 100 g oral glucose tolerance test, HbA1c, lipid profile, fasting serum insulin, HOMA-IR and fasting serum osteocalcin level.
Results
Serum osteocalcin was lower in gestational diabetes mellitus women (3.70 ± 1.55 ng/dl) than healthy pregnant women (16.61 ± 5.30) ($P<0.001$). There was a highly significant negative correlation between serum osteocalcin level and fasting blood glucose, 2 h postprandial blood glucose, HbA1c, fasting serum insulin, HOMA-IR, total cholesterol, serum triglycerides, LDL-cholesterol and highly significant positive correlation with HDL-cholesterol ($P<0.001$).
Conclusion
Serum osteocalcin level may have a role in glucose homeostasis in gestational diabetes mellitus.

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P227

Short term calorie restriction: effects on endocrine markers of nutritional status

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Calorie restriction (CR), a form of dietary regime which reduces caloric intake below the habitual unrestricted food intake is a strategy to control weight. The benefits associated with CR are numerous but the associated constant feeling of hunger makes adherence to the CR regime difficult. In the present study certain appetite regulatory hormones and endocrine markers during CR were studied. Male Sprague–Dawley rats were randomly divided into control and test groups ($n=12$ rats/group) where the control rats were *ad libitum* fed and experimental rats were kept on a 25% CR diet for a period of 5 days. The regulatory hormones and peptides i.e. ghrelin, leptin, CCK, NPY, insulin, IGF1, corticosterone, adiponectin and thyroid hormones were estimated in plasma at the end of the experiment. Decrease in body weight was 6.8% in the test group while there was a gain in body weight by 5.3% in the control rats over the 5-day period. Plasma levels of ghrelin and NPY were found to be significantly higher ($P<0.05$) in comparison to control while leptin was below the limit of detection. Insulin was not affected significantly in the CR diet in the test group. A small decline in adiponectin was found in the CR group. Partial food restriction caused a slight reduction in plasma CCK and significantly decreased IGF1 levels. Low levels of circulating T_3, as a result of energy deficit with elevated blood T_4 levels were found. The findings of the present study could be used for formulation of high satiety low calorie diets for control of metabolic disorders due to over nutrition.
Declaration of funding
Defence Institute of Physiology and Allied Sciences, DRDO, Min. of Defence, Delhi, India.

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P228

The development of a structured education programme to improve cardiovascular risk in women with polycystic ovary syndrome (SUCCESS Study)

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Introduction
Polycystic ovary syndrome (PCOS) has a prevalence of 10–15% and is associated with metabolic and mental health consequences. There is evidence that structured education programmes improve illness perception, quality of life and the metabolic profile in other chronic conditions however evidence for structured patient education in PCOS is lacking.
We aimed to use an iterative cycle of research to develop a pragmatic educational intervention tailored for the needs of women with PCOS.
Methods
In line with the Medical Research Council's framework for developing and evaluating complex interventions and using Bartholomew's intervention mapping protocol we developed an education programme for women with PCOS.
Research question was explored by literature review, consultation with peers as well as qualitative patient interviews. A semi-structured topic guide was used in the interviews to assess their need and views on the education programme and its design and content. The programme was also informed by the related literature, phenotypic and outcome analysis of a large local database of women with PCOS and the evidence from education programmes in other chronic conditions such as diabetes.
Results
The developed programme was piloted in a group of women with PCOS. Their feedback was sought through a semi-structured focus group interview. Refined intervention was piloted again in a fresh patient group and their feedback was sought.
After final refinement of the programme a group of skilled health care professional with the knowledge of the underpinning philosophies and learning theories were trained to deliver this education programme.
This pilot work has resulted in a randomised Controlled trial to test its feasibility and efficacy.
Discussion
Women with PCOS are at increased risk of diabetes, cardiovascular and mental health issues and need an early lifestyle intervention. A successful outcome of our research programme will be a milestone in treatment of this condition and improving patient care especially in primary care.
Declaration of funding
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P229

Hypogonadism in males with type 2 diabetes mellitus

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Background and objective
There have been reported increase prevalence of hypogonadism in diabetic men compared to age matched non-diabetic men. The objective of this study was to assess the level of androgens in T2DM patients and non-diabetics and correlate it with erectile function and visceral adiposity.
Methods
This was a cross-sectional comparative study of 160 male patients with T2DM (study subjects) and 80 age matched non-diabetics (control subjects). Level of free testosterone and LH was assayed, erectile dysfunction was assessed with IIEF-5 questionnaire and clinical data of both the study and control subjects were analysed.
Results
The study and control subjects were well matched for age, with mean $\pm$ S.D. of study subjects being 58.2 ± 10.1 years and control subjects was 56.6 ± 11.3 years. The study subjects had a significantly lower mean $\pm$ S.D. free testosterone level than the control subjects (16.9 ± 6.7 pg/ml compared with 21.6 ± 8.9 pg/ml, $P = 0.00$). The mean $\pm$ S.D. LH level of the study subjects was significant lower, compared to that of the control subjects (5.3 ± 3.1 mIU/l compared to 7.8 ± 5.5 mIU/l, $P < 0.05$). Among the study subjects, ED was present in 118 (73.8%) persons compared to 32 (40.0%) persons in the control subjects. The difference in the prevalence of ED in the two groups was statistically significant. ($\chi^2 = 25.9$, df $= 1$, $P = 0.00$). The mean $\pm$ S.D. BMI, WC and WHR was also statistically significantly higher in the study subjects compared to control subjects.BMI (26.2 ± 4.0 kg/m^2 compared to 24.5 ± 3.0 kg/m^2; $P < 0.001$). WC (98.7 ± 13.1 cm compared to 90.5 ± 10.5 cm; $P < 0.001$). WHR (1.0 ± 0.0 compared to 0.9 ± 0.1; $P = 0.02$).
Conclusion
Our study showed that patients with T2DM had hypogonadotrophic hypogonadism compared to the non-diabetics. Erectile dysfunction and visceral adiposity was more prevalent in men with T2DM compared to age-matched non-diabetics.
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P230

Obesity-related hypogonadotrophic hypogonadism: recovery of normal pituitary–gonadal axis function following bariatric surgery

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Background
Functional hypogonadotrophic hypogonadism (FHH) occurs in the context of any chronic disease including obese patients with type 2 diabetes (T2DM) and/or metabolic syndrome. FHH is reversible with resolution of the underlying disease process. Reported benefits of bariatric surgery include improvements in lipid profile, blood pressure and resolution of T2DM. Here we report reversal of FHH and T2DM with bariatric surgery-associated weight loss.
Case history
A 47-year-old man was referred with sexual dysfunction. He underwent puberty aged 14. His mood was low, he drank alcohol to excess, was obese (BMI 43), with sparse body hair; testes 15 ml. Baseline biochemistry confirmed hypogonadotrophic hypogonadism: LH 1.7 and FSH 4.4 IU/l; testosterone 7.2 nmol/l (NR: 9–25 nmol/l) and DEXA showed L1-4 spine osteoporosis, but serum ferritin and MRI pituitary were normal.
He was started on testosterone undecanoate 1 g i.m. (3-monthly) with symptomatic and biochemical improvement. However, he eventually became polycythaemic (peak Hb and haematocrit 18.1 g/dl and 55%, respectively) and treatment thus had to be discontinued. Shortly afterwards, he developed T2DM and, in view of his co-morbidities, was referred for lifestyle change (including counselling) and went on to undergo bariatric surgery. Post-operatively, his BMI fell to 30.3, T2DM resolved and hypertension improved. Significantly, FHH had also resolved (LH 3.1 and FSH 4.4 IU/l; testosterone 12.1 nmol/l).
Conclusion
FHH represents a physiologic response to ill health and thus may serve as a useful biological function. Several short-term interventional studies have demonstrated improvements in various surrogate endpoints with testosterone therapy in patients with FHH in relation to obesity and/or metabolic syndrome. Nevertheless, given the paucity of longitudinal safety/efficacy data, it may be more appropriate to target the underlying problem of obesity, rather than opting for testosterone replacement. As demonstrated in our patient, weight loss resulting from bariatric surgery can lead to recovery of pituitary–gonadal axis obviating the requirement for testosterone replacement therapy.
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P231

Risk assessment of adult residents in ile-ife, South-Western Nigeria for type 2 diabetes mellitus

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Background
Type 2 diabetes mellitus (T2DM) is a potentially preventable disease that is presently increasing in epidemic proportion worldwide. Its onset can also be delayed with an appropriate and timely intervention if people at risk are identified early. This study determined risk levels of adults in Nigeria for type 2 DM by using Finnish Diabetes Risk Score (FINDRISC).
Method
During our local activities for world diabetes day in 2011, adults who were not previously known to be diabetic voluntarily underwent screening examination after an overnight fast at OAUTHC, Ile-Ife. Each participant had their anthropometry, blood pressure, fasting plasma glucose (FPG) measured and FINDRISC assessed.

Results
There were 158 participants, 85 (53.8%) males and 73 (46.2%) females. Majority, 80 (50.7%) were in age group 45–64 years. 10 (6.3%) subjects had high risk with a total risk score of 15–20 which estimated that one in three subjects will develop diabetes within 10 years. None of the subjects had very high risk. Only 142 (89.9%) subjects had FPG done. High risk subjects had 4 (40.0%), 1 (10.0%) and 5 (50.0%) of them with normal FPG, impaired FPG and diabetic FPG respectively. There was a significant association between the type 2 diabetes risk levels and FPG ($P=0.014$) and SBP ($P=0.008$).
Conclusion
The Finnish Diabetes Risk Score is a useful noninvasive method of screening in our environment. We recommend its use as a self-administered questionnaire in our population so that individuals will know their risk levels early and adopt appropriate life style modifications.

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P232

Adrenal insufficiency post bariatric surgery

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Introduction
NICE recommends weight loss surgery as a treatment option for people with obesity. However, long term data on outcomes and complications on surgery are limited. We report unexplained adrenal insufficiency post bariatric surgery.
Case report:
Patient one
27 years old lady underwent Roux-en-Y gastric bypass (RYGB). Weight loss (kg) was from 130.2 to 73.2 over a 2-year period. Patient complained of general unwell and dizziness. No orthostatic hypotension was noted. Random cortisol (nmol/l) was low at 98 and Short synacthen test (SST) showed 0 and 30 min Cortisol of 158 and 386 respectively with a low ACTH.
Patient two
46 years old man underwent RYGB. Weight loss was from 151.8 to 56.8 over a period of 18 months. He complained of episodes of vomiting, sweating and black outs with no postural hypotension. Random cortisol was 50 and SST demonstrated cortisol of 108 and 281 at 0 and 30 min respectively. Steroid replacements were commenced and he improved symptomatically.
Discussion
The cause of adrenal insufficiency in the above cases remains unexplained. Adrenal insufficiency has been reported after major surgery as result of stress or blood loss affecting the pituitary gland. However only one case has been reported post bariatric surgery. Possible mechanisms are malabsorption of bile affecting cholesterol leading to reduced precursor for steroid synthesis, malabsorption of trace elements and vitamins (especially selenium and vitamin B5) that are steroid biosynthesis cofactors, re-setting of hypothalamo-pituitary–adrenal axis due to weight loss as in anorexia nervosa and perioperative complications such as blood loss causing pituitary/adrenal infarct or apoplexy.
Conclusion
These two cases suggest the importance of patient selection, pre surgical counselling and long term follow-up post bariatric surgery.

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P233

Pregnancy outcome in a patient with lipodystrophy and type 2 diabetes

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Background
A 23-year-old woman diagnosed with Dunnigan-type familial partial lipodystrophy (FPLD) attended the joint Antenatal/Endocrine Clinic at 13 weeks of gestation. She was diagnosed at age 7 and subsequently developed type 2 diabetes at age 11 years. She was managed initially with metformin followed by addition of insulin. It was an unplanned pregnancy and her booking HbA1c was 8.4% (IFCC 68 mmol/mol).
Prior to pregnancy, she was on a basal bolus regime of Levemir and Novorapid and her total daily insulin dose was ~180 units. At booking, we added Metformin 500 mg twice daily, which was later increased to three times daily.
Her insulin requirements escalated rapidly to a total daily dose of 250 units by 25 weeks gestation. At this stage, the use of Humulin R (U500) was considered and discussed with the patient. However, she presented with vaginal spotting and went into spontaneous labour at 26 weeks. She delivered a live female infant who was admitted briefly to the special care baby unit. During labour she was managed with intravenous sliding scale insulin.
Conclusion
Familial Lipodystrophy is a group of rare disorders associated with numerous metabolic complications. Diabetes, familial lipodystrophy and pregnancy in combination all compound and confer a severe insulin resistant state which if poorly controlled, can have an adverse effect on pregnancy outcome. Data on the use of U500 insulin during pregnancy is limited however there are a handful of case reports of its use in pregnancy with successful management of glycaemic and obstetric outcome.
This case is a reminder of the challenges in the glycaemic management of patients with lipodystrophy particularly in pregnancy. It also adds to the limited literature available on pregnancy outcome of patients with lipodystrophy.

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Impaired iron status in severely obese bariatric surgery candidates is multifactorial

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Background and objectives
Obesity is associated with a relatively high prevalence of anaemia and iron deficiency compared to normal weight individuals; the cause is unknown. We aimed to determine the prevalence of iron deficiency and anaemia in a severely obese cohort of bariatric surgery candidates, and to explore underlying associations with markers of nutrition and inflammation.
Methods
In a retrospective review of 703 consecutive patients presenting for bariatric surgery assessment, 656 were evaluated after exclusions for haemoglobinopathy and missing data. Assessment included; clinical history, basic anthropometry, and blood tests (haemoglobin (Hb), serum iron, total iron binding capacity, iron binding saturation, ferritin, vitamin B_1, vitamin B_{12}, folate, white cell count (WCC), and C-reactive protein (CRP)). Anaemia was defined as; men: Hb < 13 g/dl, or women: Hb < 12 g/dl; and iron deficiency as an abnormality in two or more measures of iron status. Indirect measures of adiposity included BMI and percentage body fat (BF%) estimated from the CUN-BAE equation. Statistical analyses: stepwise regression was performed with independent predictors of serum iron concentration: age, gender, BMI, BF%, index of multiple deprivation, diabetes status, number of comorbidities, WCC, CRP, folate, vitamin B_{12}, and use of multivitamins, metformin, insulin, non-steroidal anti-inflammatory drugs (NSAIDs) or proton pump inhibitors.
Results
Anaemia and iron deficiency were present in 9.9 and 11.4% of patients, respectively. Stepwise regression revealed an association between serum iron concentration and CRP ($R^2=0.227$; $P=0.001$), however CRP concentrations were only available for 156 patients (24%). Excluding CRP from analyses, WCC had the largest effect on serum iron concentration, with NSAIDs, gender, and diabetes status also contributing ($R^2=0.076$; $P<0.001$), albeit leading to a much weaker association than observed with CRP.
Conclusion
Anaemia and iron deficiency are relatively common in severely obese individuals, potentially due to inflammation causing deranged iron homeostasis, although a multifactorial aetiology is likely.

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P235

What lies beneath: a case of spontaneous hypoglycaemia or glucose transporter type 1 defect disguised as chronic fatigue?

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A 42-year-old female had extensive neurological investigations (normal MRI brain, EEG, NCS). A low CSF glucose triggered endocrine referral. She had a history of ill health/fatigue since 19 years when she had a viral illness with seizure-like episode and transient wheelchair dependance, with no formal diagnosis made. She had recurrent symptoms including fatigue, myalgia and

weakness, with some relation to hunger and fasting.
Physical examination revealed macrocephaly, mild generalised reduction in power and ataxia, with brisk bilateral lower limb reflexes.
A full fatigue screen was normal. Prolonged fasting led to symptoms at 24 h of fatigue, left calf pain and multiple episodes of rhythmic left leg jerking lasting approximately 20 s, during which she was fully conscious. Clinical examination demonstrated left hemiparesis (4/5) and reduced sensation. Lab glucose was 4.3 mmol/l, plasma lactate 0.9 mmol/l (0.5–2.2) during the episode. She declined further fasting.
She was referred to the regional adult inherited metabolic disorders team. The history and examination is characteristic of the paroxysmal late-onset form of glucose transporter type one (GLUT 1) deficiency syndrome. This is usually caused by mutations in the SLC2A1 gene and results in impaired glucose transport into the brain. Patients develop classical features of hypoglycaemia with normal peripheral blood glucose. Some marginal changes may occur in serum lactate (i.e. inappropriately low <1 mmol/l) when symptomatic. Lower limb pain and jerking is characteristic. Ketogenic diet is effective in 60% of cases. This is an autosomal dominant condition and is likely to be under-diagnosed. Cases may appear in endocrine clinics because of hypoglycaemic symptoms and hunger. Symptoms may be assumed to be functional or related to chronic fatigue syndrome.

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P236

Improvement in testosterone post bariatric surgery

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Introduction
Obesity is known to be associated with hypogonadotropic hypogonadism. Hypogonadism is an established risk factor for cardio vascular disease and type 2 diabetes mellitus (T2DM). However there is little evidence on improvement in testosterone with bariatric surgery.
Aim and methods
To asses changes in testosterone levels after bariatric surgery. Retrospective study on men undergoing bariatric surgery, and data collected pre and post surgery. Data was analysed for normality followed by paired Wilcoxon signed rank tests. State version ten was used for analysis.
Results:
22 patients underwent bariatric surgery and were followed up over a median interval of 11 months. The mean (s.d.) age was 49.49 (8.55) years. Mean pre operative weight (kg) was 151.09 (23.88). Mean weight loss was 35.5 (12.1–74). There was significant rise in total testosterone levels, mean Pre op and post op testosterone (nmol/l) being 9.02 (3.66) and 14.5 (9.04) respectively ($P=0.009$). 50% of patients in study group showed improvement in their testosterone level. FSH and LH levels improve after surgery though these were not significant. Linear regression models showed that the rise in testosterone was not determined by weight loss.
Discussion
Testosterone levels are inversely proportional to the BMI. There are several mechanisms by which obesity can cause hypogonadism.In the above study, as only half of them showed improvement in testosterone and not that all the patients came off testosterone after surgery. This indicates that there may be uncertain mechanisms apart from weight loss that improves the level.
Conclusion
This is one of the studies on improvement in testosterone level after weight loss. Further large scale data is needed to look into the long term effect of weight loss in sex hormones and also to identify other uncertain mechanisms which improves the testosterone.

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P237

Prevalence and associated risks for metabolic syndrome in nigerians with type 2 diabetes mellitus

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Introduction
Diabetes mellitus is considered a cardiovascular risk equivalent. The presence of other cardiovascular risk factors in patients with diabetes may imply synergy for the occurrence and severity of cardiovascular morbidities and mortalities. Metabolic syndrome is the presence of these clusters of cardiovascular risks in an individual. Metabolic syndrome has been shown to be associated with some traditional risk factors and emerging risk factors.
Aim
This study determined the prevalence of metabolic syndrome defined by the International Diabetes Federation (IDF) in people with type 2 diabetes mellitus, and also determined its associations.
Method
A case–control study involving 150 Nigerians with Diabetes and 150 age and sex matched controls. Relevant clinical and anthropometric measurements were taken and laboratory investigations were done. Statistical analysis was done using SPSS 14. Regression analysis was done to determine the factors associated with Metabolic Syndrome and regression results were interpreted using odds ratio and confidence intervals.
Results
The mean $\pm$s.d. age of the diabetic and control groups were 56.12 ($\pm$7.65) years and 55.76 ($\pm$7.49) years respectively ($P=0.681$). Prevalence of metabolic syndrome among diabetics and control were 68.7 and 14.7% respectively. Female gender, hypertension, and abdominal obesity were associated with increased odds for metabolic syndrome.
Conclusion
Metabolic syndrome is common among our diabetic patients. Detection and management of its cluster of risks may be an essential component in reducing its occurrence.

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P238

Ethnic specific anthropometric values have been used by the International Diabetes Federation to aid in the diagnosis of the metabolic syndrome: no such values are available for Sub-Saharan Africa including Nigeria

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Objectives
To determine the mean and normative values of anthropometry among the inhabitants of a coastal Nigerian city.
Methods
A cross sectional survey comprising 1134 subjects (645 males and 489 females) representative of the entire population of Calabar metropolis aged 15–79 was studied. A multistage sampling method was applied to select the subjects. Using a modification of WHO STEPS instrument the information obtained included anthropometric indices. Anthropometric indices were expressed as mean (s.d.). The comparison of means between groups was done using independent student *t*-test. The normative values of indices of nutriture were determined using CI, and the level of significance was taken as $P<0.05$.
Results
The mean (95% CI) values of BMI for males and females were 27.0 kg/m^2 (95% CI 26.5–27.2) and 28.5 kg/m^2 (95% CI 28.0–29.0) respectively. The mean (95% CI) value of waist circumference (WC) for males was 91.0 cm (95% CI 90.2–91.8), of females was 89.8 cm (95% CI 88.8–90.8). The mean (95% CI) values of waist hip ratio (WHR) for males was 0.90 (95% CI 0.88–0.89) while that of females was 0.85 (95% CI 0.87–0.89). The mean (95% CI) values of height for males was 1.70 m (1.70–1.72) and for females was 1.60 m (1.64–1.65), $P<0.01$. The mean (95% CI) values of hip circumference (HC) for males was 101.1 cm (101.5–103.3) and for females was 105.6 cm (102.8–104.4), $P<0.01$.
Conclusion
The mean values of anthropometry in the study population are different from those from other parts of Nigeria and other parts of the world.

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P239

Preoperative characteristics of morbidly obese patients who achieved at least 50% excess weight loss post-metabolic Surgery

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Background
Pre-operative predictive measures of post-metabolic surgery success would facilitate a more refined evidence-based selection of patients. This study aimed at identifying those clinical and psycho-social predictors of success (defined as weight-loss) following metabolic surgery.
Methods
Pre-operative data including IWQOL-Lite questionnaire (a validated self-reported 31-item measure of physical function, self-esteem, sexual life, public distress and work related domains of obesity-specific quality of life (QOL)) scores transformed to a 0–100 scale, where a score of 100 represents the best health-related QOL (HRQOL), were obtained for 26 patients who had completed 1 year follow-up post-metabolic surgery at University Hospitals Coventry and Warwickshire. Comparisons were made between pre-operative characteristics from two subgroups: patients with <30, and $>50\%$ excess weight lost 1 year post-operatively (towards an ideal BMI 25 kg/m^2 (EWL-1 year)). Statistical analyses were done for pre-operative variables and EWL-1 year. Results are reported as (mean) and (S.D.). $P<0.05$ is considered statistically significant.
Results
Comparisons between the subgroups with lower and higher EWL-1 year values ($n=12$ and 9 respectively) showed no significant differences in pre-operative HRQOL scores for all QOL domains. Only pre-operative BMI and EWL-1 year had significant correlation ($r=0.499$, $P=0.009$). Pre-operatively, BMI showed a significant negative correlation with public distress HRQOL ($r=-0.54$, $P=0.005$) and self-esteem related HRQOL ($r=-0.409$ $P=0.041$). Multivariate linear regression analysis with age, sex, BMI and HRQOL scores as independent variables and EWL-1 year as a dependent variable, revealed that pre-operative BMI had a significant independent association with EWL-1 year ($\beta=0.70$, $P=0.01$).
Conclusion
We show a significant, independent and direct relationship between pre-operative BMI and excess weight-loss one year following metabolic surgery, although pre-operative QOL measures were not predictive of EWL-1 year. It remains possible that pre-op QOL may predict improved psycho-social function post metabolic surgery.

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P240

Glycaemic variability: does it make a difference in prediabetes?

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Introduction
Glycaemic variability has been proposed as a contributing factor for development of diabetes related complications. This concept originated from Epidemiology of Diabetes Interventions and Complications (EDIC) study, in which, although the HbA1C was similar in conventional and intensively treated groups, the incidence of retinopathy was higher in the conventional group. This was attributed to glycaemic variability. Studying glycaemic variability in prediabetes would provide a better understanding of prediabetes and its complications.
Aims and objectives
To study glycaemic variability in prediabetic Indian women.
Materials and methods
After informed consent, prediabetic women (based on OGTT) were connected to a continuous glucose monitoring (CGM) device for 72 h. Various indices of glycaemic variability including mean amplitude of glycaemic excursions (MAGE) were calculated using the easy GV software©.
Results
Fifteen eligible women were enrolled over a period of 6 months. The mean age was 36.13 years (S.D. 9.1) and mean BMI was 31.54 kg/m^2 (S.D. 7.86). All subjects had interstitial glucose measurement <200 mg/dl during the 72 h of CGM recording. The mean MAGE was 2.74 (S.D. 0.72). MAGE was elevated (>1.3) in 47% of the subjects. Individuals with prediabetes had different glycaemic variability irrespective of their subgroup (IFG/IGT/both). There was no significant correlation between MAGE and BMI, HbA1c or triglycerides. There was a trend towards positive correlation of MAGE with waist circumference ($R=0.527$, $P=0.053$).
Conclusions
Women with prediabetes showed a range of glycaemic variability across the board irrespective of their subgroup (IFG/IGT/both), thus providing an opportunity to categorize prediabetics in a distinct way. A prospective study would be required to see whether the categorization based on glycaemic variability has the ability to stratify risk and has treatment implications.

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P241

Unusual case of hypoglycaemia in diabetic patient

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A 73-year-old lady with a history of type two diabetes since 1993, mastectomy for carcinoma left breast in 2000, primary hypothyroidism, and hypertension was referred to the local hospital in view of poor glycaemic control in July 2001. Her medications included Thyroxine, Atenolol, and Tamoxifen, Frusemide, Gliclazide 160 mg BD and Pioglitazone 30 mg. At the time of referral her weight was 68.5 kg, (BMI 23.7), HbA1c 10.2%. She was started on Humalog mix 25 BD. In 2003 her weight went up to 83.3 kg with HbA1c of 7.8%. In 2004 she was started a basal bolus regime with Humalog TDS and Glargine. In 2010 her weight increased to 93.9 kg (BMI 32.5) with HbA1c of 8.5%. She became depressed with 25 kg weight gain over a period of 9 years and therefore started on an antidepressant in September 2011. When reviewed in January 2012 her weight was down to 89 kg with a better HbA1c of 7.0% without changes in her diet, physical activities and renal function. She was having hypoglycaemias. She sensibly cut down the insulin doses. Now she is only on Glargine 40 units at night. The cause of hypoglycaemia was investigated including the short synacthen test which was normal. Apparently she was commenced on Citalopram by the GP for 12 months.
Conclusion
Patients with type two diabetes are at a higher risk for depression. Selective serotonin reuptake inhibitors (SSRIs) are effective anti-depressant. Hypoglycaemia in insulin treated diabetic patient is common. Taking a thorough drug history is essential especially Citalopram when presented with hypoglycaemia with no apparent reason. About 1% of people taking Citalopram developed hypoglycaemia. SSRIs do not seem to influence plasma insulin levels or augment hypoglycaemic action of injected insulin.

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P242

withdrawing insulin in a young person

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On reviewing patients who are on Mixtard 30, We came across a 17-year-old Asian boy who was diagnosed to have type 1 DM and learning difficulties. We noted that his HbA1c was 5.5. According to notes he was on 16 and 8 units of Mixtard 30. We discovered that he has not been taking his evening insulin (other than a short period after diagnosis) because he was having low BM. His diet consist of burgers, orange juice, takeaways and some coke.
We have been gradually working on his diet and reducing his morning insulin. On 29/10, We have stopped his insulin completely. He is under close follow up and remains Insulin free to date.
His story goes
Since the 2nd day of his life, he was found to be unduly sleepy with low blood sugars despite treatment with dextrose, hourly feeds and glucagon injection 6 h. Blood taken during a hypo episode: insulin levels 305 C-peptide 1565.
U/S scan of the pancreas– cystic mass in the tail of pancreas.
– Subtotal pancreatectomy (Tail, body, head of pancreas removed).
Pathology report – Features consistent with nesidoblastosis
July 2005 – HbA1c – 6.0. Feb 06 – School nurse did some BM incluiding ones up to 20. In the clinic, HBAIC – 8.6.Patient had no symptoms of thirst, polyuria or nocturnal enuresis. Ketone testing was negative. Mix 30 – 22-morning/11-evening was started.
April 06 – HbA1c – 7.6 Nov 06 – BM ranged between 5–9.
Since then Hbaic – Aug 08 – 5.2, Aug 09 – 5.4, Jan 10 – 5.5, Sept 10 – 5.5.

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P243

Re-occurrence of pancreatic insulinoma: an usual cause of hypoglycaemia

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A 42-year-old woman presented to her GP with episodes of feeling 'shaky' exacerbated by physical exercise and prolonged fast. She was previously diagnosed with an insulinoma in 2006 (serum glucose 1.6 mmol/l, serum insulin 3.1 mIU/l and serum C-peptide <165 pmol/l). CT abdomen /transabdominal ultrasound revealed a 1 cm insulinoma in the uncinate process of the pancreas and the patient later underwent pancreatic enucleation in 2006. Post pancreatic enucleation, 72 h fast was negative. The patient remained asymptomatic *post* op and re-presented to a locum GP 6 years later with initial symptoms. She was reviewed during her annual follow-up and due to concerns relating to her background she was admitted to the specialist endocrine department for further investigations. A 72 h fast was positive for hypoglycaemia with serum glucose level 1.8 mmol/l, serum insulin 8.6 mIU/l and serum C-peptide 443 pmol/l.

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Pituitary

P244

Secretory granule accumulation in anterior pituitary somatotrophs of TPC1 null mice

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Calcium mobilization from intracellular stores represents an important cell signalling process that is regulated, in mammalian cells, by inositol-1,4,5-triphosphate (IP_3), cyclic ADP ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP). Intracellular calcium is important for mobilization of secretory granules to the plasma membrane in preparation for exocytosis. NAADP mobilizes calcium from lysosome-related acidic compartments and it has been shown that two-pore channels (TPCs) comprise a family of NAADP receptors, with TPC1 expressed on endosomal membranes. Western blot analysis and RT-PCR have revealed TPC1 expression in anterior pituitary and double-labelling immunofluorescence labelling for growth hormone GH and TPC1 demonstrated colocalisation in somatotrophs. Here we test the hypothesis that GH secretion in somatotrophs from TPC1 null mice would be impaired and excess storage of secretory granules would result. Anterior pituitary sections from male and female wild-type (WT) and TPC1 null mice ($n=4$ of each) were immunogold labelled for GH and examined by quantitative electron microscopy to determine somatotroph size, secretory granule characteristics and distribution. In female TPC1 null mice there was a significant ($P<0.01$ vs WT) increase in cell and cytoplasmic area, and a significant increase ($P<0.01$) in granule density suggesting increased synthesis and storage of GH. Furthermore, there was a decrease in the percentage of secretory granules within a 300 nm margin of the plasma membrane indicating that fewer granules were distributed adjacent to sites of secretion ($P<0.01$). In male TPC1 null mice there was no significant difference measured in cell and cytoplasmic area, granule distribution or size but a significant ($P<0.05$ vs WT) increase in granule density was measured. In conclusion, the data are consistent with increased GH storagel in the absence of TPC1.

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P245

Transport features of pituitary folliculostellate cells increase in pregnancy

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Folliculo-stellate (FS) cells exert a paracrine regulation on their neighbouring endocrine cells in the anterior pituitary gland. FS cells are non-granular cells characterized by long cytoplasmic processes and form follicles with microvilli on their luminal cavity, suggesting a transport function. Moreover, FS cells form monolayers in primary culture and develop domes after reaching confluence, characteristics of polarized transport epithelia. However little is known about transporter proteins in FS cells. We investigated the expression of three transporters: the peptide transporter PepT2, the glucose transporter 2 (Glut2) and the cholesterol and annexin one transporter ATP-binding cassette transporter A1 (ABCA1) in two FS cell lines (TPit and TtT/GF cells), CaCo2 cells (a positive control epithelial colorectal cell line) and anterior pituitary by immunogold electron microscopy and western blotting. Immunogold particles for each of the transporters investigated were detected in Caco-2 cells. TPit, TtT/GF and anterior pituitary cells were found to express ABCA1, Glut2 but not PepT2 and the same findings were obtained in FS cells in rat anterior pituitary. In response to changing physiological demands during pregnancy, the pituitary has the ability to expand its cell number several fold and FS cell transport systems may be important for supporting these changes. Therefore FS cell size and ultrastructure were studied by use of electron microscopy in virgin and pregnant (1 and 3 weeks) rats. FS cell size was significantly increased in 3 weeks pregnant rats ($P<0.01$) with more numerous microvilli compared to virgin rats and an increased number ($P<0.01$) of FS cells were located adjacent to blood. In weaned rats FS size was not significantly different to virgin. An increase in Glut2 immunogold labelling of FS cell microvilli was measured in 3 weeks pregnant pituitary consistent with a role for FS cells in supporting increased nutrient requirements for pituitary growth in pregnancy.

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P246

Chronic glucocorticoid exposure causes *de-novo* methylation of genes key to the regulation of the hypothalamic–pituitary–adrenal axis

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Introduction:
The HPA axis is essential for mammalian life. Proopiomelanocortin (POMC), expressed in corticotroph cells of the pituitary, is the master activator of the axis, and a classical negative feedback loop exists whereby glucocorticoids from the adrenals repress its expression. Glucocorticoids are commonly prescribed medicines (10 million prescriptions in UK per year), but when used long-term, suppression of the HPA axis is a major side effect, with risk of life-threatening adrenal failure even after withdrawal of treatment.
Hypothesis:
Glucocorticoids induce *de novo* DNA methylation of *POMC* or *POMC*-activating transcription factors, accounting for long-term inhibition even after withdrawal of treatment.
Methods:
Methylation patterns were studied by bisulphite sequencing in murine ACTH-expressing (AtT20) and non-expressing (3T3-L1) cell lines, before and after long-term culture in dexamethasone or vehicle. Gene expression was assessed by

qPCR.
Results:
Expression of *POMC* and *Tpit* was dramatically reduced in AtT20 cells, whereas that of NeuroD1 and Pitx1, two *POMC*-activating transcription factors, was not. Dexamethasone treatment was associated with *de-novo* methylation of the *Tpit* promoter region, which was methylated in 3T3-L1 cells under normal culture conditions. *Tpit* and *POMC* expression was rescued by the histone deacetylase inhibition.
Conclusion:
To our knowledge this is the first example of *de novo* DNA methylation induced by glucocorticoids. These data may have important implications for use of epigenetic modifying agents after chronic glucocorticoid therapy to allow recovery of the HPA axis.

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P247

Polymersomes-mediated siRNA delivery for states of hormone excess

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Introduction
Cushing's disease is a devastating condition associated with a fivefold excess mortality. It is usually due to a small (few mm) benign corticotroph tumour in the pituitary expressing excess pro-opiomelanocortin (POMC), the peptide product of which, ACTH, drives excess secretion of cortisol from the adrenal. There is a clear clinical need for better treatment options.
Background
We have designed, optimized and validated unique siRNAs to POMC and shown highly effective and durable knockdown *in vitro* and *in vivo.* Here, we have extended these data to assess the effectiveness of polymersomes, which are biomimetic and polymer-based vesicles, for enhanced delivery of anti-*pomc* siRNA.
Methods
Polymersomes were formed using the amphiphilic, pH sensitive, PMPC (poly (2-(methacryloyloxy) ethyl phosphorylcholine) – PDPA poly (2--(diisopropylamino)ethyl methacrylate) copolymers. Effectiveness of polymersomes-mediated siRNA delivery was studied in the AtT20 cell line.
Results:
Polymersomes are effective for the delivery of siRNA, supporting their application to deliver anti-*pomc* siRNA as therapy.
Conclusion
These data further support the potential of a novel epigenetic therapeutic approach for Cushing's disease.

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P248

Transcriptional regulation of C-type natriuretic peptide (CNP/Nppc) and its receptor guanylyl cyclase-B (GC-B/Npr2) in gonadotroph and somatolactotroph cell lines

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C-type natriuretic peptide (CNP) has recently been implicated as a key meiotic arrest factor in oocytes, and mechanistic studies suggest that the transcriptional regulation of the CNP gene (Nppc) and of its receptor, GC-B (Npr2) is sensitive to gonadotrophin-dependent cAMP accumulation. We have shown CNP to be a major regulator of gonadotrophs in the pituitary, but have yet to establish how either Nppc or Npr2 are transcriptionally controlled locally. In the current study, the Nppc promoter, spanning -1209 to +56 relative to the transcriptional start site was cloned into pGL3LUC from a BAC containing mouse Ch1. αT3-1 and GH3 cells were transiently transfected with Nppc-LUC plasmid, treated with the adenylyl cyclase activator, Forskolin (FSK, 10^{-9}–10^{-5} M) for 24 h, after which whole cell lysates were collected and luciferase reporter assays performed. A dose dependent increase in Nppc promoter activity was observed with increasing concentrations of FSK in αT3-1 cells (1.5±0.5–8.0±3.5-fold increase compared with baseline), with a similar pattern observed in GH3 cells (1.4±0.1–3.4±1.6-fold increase), although maximal activity was seen at 10^{-6} M (3.9±0.7-fold increase). To establish whether cAMP signalling could also affect the expression of the CNP receptor, GC-B, similar experiments were performed using a reporter construct containing the human NPR2 promoter, spanning −2129 from the transcriptional start site (NPR2-LUC). Interestingly, FSK failed to stimulate the NPR2-LUC promoter in αT3-1 and GH3 cells, and surprisingly caused an apparent inhibition at lower concentrations (33.9±10.0–26.7±7.5%, 18.4±3.8–23.2±4.6% respectively). In summary, these data suggest that cAMP signalling can differentially alter transcriptional regulation of Nppc and Npr2 in pituitary cell lines, providing some parallels with the regulation of these genes in ovarian tissues. However, the specific mechanisms involved in these transcriptional effects remain to be established.
Declaration of funding
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P249

'Invasion signature' revealed by the analysis of AIP positive and AIP mutation negative human pituitary adenomas

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Background
Familial isolated pituitary adenoma (FIPA) is an autosomal dominant condition with incomplete penetrance. Heterozygote mutations have been identified in the aryl-hydrocarbon receptor interacting protein (AIP) gene in 20% of FIPA families causing young-onset aggressive tumours.
Aims
The aim of this study was to perform comparative gene expression microarray analysis of familial AIP positive and AIP negative adenomas and compare them to sporadic tumours and normal pituitary to discover novel genes and pathways responsible for familial pituitary tumorigenesis.
Methods
We have performed gene expression analysis on normal pituitary, sporadic GH-secreting adenomas, AIP positive and AIP negative familial somatotroph adenomas (five samples of each category) using the Affymetrix human Gene Chip HG-U133 Plus 2.0 array. Data analysis was carried out in the statistical 'R' environment. Ingenuity Pathway Analysis (IPA) tool was used for pathway analysis. Expression of the ten selected genes from microarray analysis was validated by quantitative reverse transcriptase PCR. Functional assays were performed using BioCoat-Matrigel invasion chambers.
Results
We have identified differentially expressed genes in AIPpos (451 up; 1249 down) and AIPneg (234 up; 1609 down) pituitary adenomas compared to normal pituitary. A smaller number of genes differ in their expression levels between familial AIP positive and sporadic adenomas (10 up; 22 down) and 45 genes (20 up; 25 down) in AIPpos compared to AIPneg tumours. IPA of these genes revealed one of the significantly altered functional modules: 'cellular invasion signature'. Several genes of the invasion signature have been validated by RT-qPCR. Functional studies on invasion characteristic with AIP knockdown cells support these data.
Conclusion
The observed transcriptional changes probably reflect the more aggressive clinical phenotype in AIP positive patients. The identified genes may predict the invasive potential of these tumours.
Declaration of funding

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P250

Maternal vocalisation as an effective priming method for oxytocin in young adults

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The neuropeptide oxytocin is the most recent peptide to have emerged from a new field of research investigating the physiological underpinnings of human social behaviour. Whilst previous studies have focused on the role of touch as an effective method of priming, this study investigated the role of social vocalisation

as a potential priming method of endogenous oxytocin release. 40 female participants, aged between 18 and 21 years of age, were randomly allocated into either a primed or unprimed condition. Primed participants engaged in a phone call with their mothers, whilst unprimed participants rang a cinema hotline. The study illustrated that primed participants had significantly higher oxytocin concentrations compared to unprimed participants. Interestingly, primed participants' baseline oxytocin concentrations were also higher than unprimed participants, thus there appears to be an anticipation response in primed participants. The results provide initial evidence for maternal vocalisation as an effective priming method for endogenous oxytocin in young adults.

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P251

Altitude acclimatization: plasma AVP response and physiological changes

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Background
Arginine vasopressin (AVP) changes during altitude acclimatization is of clinical interest as increases in their plasma levels (with reference to sea-level (SL)) have been associated with fluid retention accompanied by elevated plasma cortisol levels. Studies have reported no change/ decrease in plasma AVP during 'normal' acclimatization. This study was conducted to evaluate plasma AVP changes and the associated physiological changes during chronic exposure to high-altitude (HA).
Methods
Healthy, male volunteers ($n=36$) between 20–50 years of age were recruited for the study. Subjects were evaluated both at SL and HA (4500 m, 3–4 weeks of stay) for the following parameters: plasma cortisol (CORT) and AVP (ELISA), haemoglobin(Hb), hematocrit(Hct), plasma sodium (Na); total protein (PROT), body weight (BW), arterial oxygen saturation (SaO_2), heart rate (HR), respiratory rate (RR), mean arterial pressure (MAP). Values are mean ± S.D.
Results
At HA, all subjects were asymptomatic and exhibited physiological characteristics of altitude acclimatization (↓: BW, SaO_2; ↑: Hb, Hct, MAP, HR; ↔: RR). Plasma AVP and CORT level during HA exposure was within 'SL normal range'. Subjects were categorized as follows:

Table 1

		AVP			CORT			
Category AVP_{SLvsHA}	*n*	SL	HA	*P* value	SL	HA	*P* value	**PROT/Na**
I (↔)	8	0.7±0.2	0.7±0.2	>.05	10.0±3.0	8.6±3.6	>.05	↔
II (↓)	16	0.9±0.2	0.6±0.2	<.05	10.7±3.1	11.7±4.6	>.05	↑
III (↑)	12	0.6±0.1	0.9±0.2	<.05	9.9±1.9	12.3±2.8	<.05	↑/↔

Conclusions
Altitude acclimatization is characterised by physiological variation in both plasma AVP and CORT levels. However, the significance of 'subtle' changes in AVP with regard to body fluid regulation (re-setting of osmotic threshold) needs further evaluation.

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P252

Craniopharyngiomas and Wnt signalling pathways

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Craniopharyngiomas are tumours which grow in the region of the sella, with adamantinomatous (ACP) and papillary (PCP) subtypes. While usually 'benign', They can have devastating long term sequelae, both from the mass effects of the tumour itself on the visual, pituitary or hypothalamic pathways, but also from the neurosurgical challenge to achieve tumour control with preservation of the surrounding pituitary and hypothalamic pathways. To date there is no satisfactory medical therapy for these tumours. The ACP subtype accounts for 10% of paediatric intracranial tumours. Potential therapies may depend on establishing and exploiting the molecular pathogenesis of these tumours.
Key components of the Wnt signaling pathway have previously shown to play important roles in colorectal, breast, stomach and prostate cancer. Mutations in the β-catenin gene, CTNNB1, have been implicated in the tumorigenesis pathway of ACPs. β-Catenin plays a role in cadherin mediated cell-cell adhesion, and also acts as a signal mediator, functioning as a downstream mediator. It is thought to stabilise and accumulate in the cytosol, translocating to the nucleus and inducing the transcription of the Wnt target genes. Upstream it is linked to membrane cadherins such as E-cadherin, α-catenin, and plakoglobin. These interactions to date not been explored to our knowledge in craniopharyngiomas.
We have examined in a large cohort of 98 craniopharyngiomas, comprising 80 ACPs and 18 PCPs modifications in the Wnt signalling pathway, particularly in β-catenin, helping to separate them as distinct entities. β-catenin was found to be translocated into the nucleus in discrete clusters of tumour cells in all (100%) ACPs, but the gene mutation rate of the β-catenin CTNNB1 gene was only 50%. Further to this, we are currently exploring changes in other parts of the E-cadherin complex of proteins, which may account for the aberrant β-catenin localisation.

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P253

In vivo characterisation of skeletal muscle metabolism in GH deficient adults using phosphorus-31 magnetic resonance spectroscopy

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Context
GH deficient (GHD) adults can experience fatigue which resolves with GH replacement. The precise basis of this is unclear. Suboptimal mitochondrial function has been demonstrated in several conditions in which fatigue is a prominent symptom. Phosphorus-31 magnetic resonance spectroscopy (31P-MRS) can measure maximal mitochondrial oxidative phosphorylation, an important parameter of mitochondrial function. We have adapted this technique to enable non-invasive measurement of muscle mitochondrial oxidative phosphorylation *in vivo* during dynamic muscle activity.
Objective
To characterise and compare *in vivo* skeletal muscle metabolism in age, gender and physical activity matched untreated GHD adults, treated GHD adults and healthy volunteers. We also compared the perception of fatigue using specific domains within Qol-AGHDA across the three groups.
Design
Twenty two untreated GHD adults, 23 treated GHD adults and 20 healthy volunteers were recruited at a tertiary University centre. All patients underwent assessment of muscle mitochondrial function ($\tau_{1/2}$ PCr) using ^{31}P-MRS. Fasting biochemical analyses and anthropometric measurements were obtained. All patients completed questionnaires on quality of life (QoL-AGHDA) and physical activity assessment (IPAQ).
Results
There was no difference in maximal mitochondrial function ($P=0.53$) and proton handling ($P=0.30$) of skeletal muscle between untreated GHD, treated GHD and healthy volunteers. There was no association between $\tau_{1/2}$ PCr and serum IGF1 ($r=-0.13$, $P=0.32$). Untreated GHD adults complained of significantly increased fatigue and impaired QoL when compared to treated GHD adults and healthy controls ($P=0.009$, $P=0.002$). Untreated GHD patients had significantly lower IGF1 than both treated GHD and healthy volunteers ($P<0.001$).
Conclusions
Whilst untreated GHD adults experience fatigue compared to treated GHD adults and normal volunteers, they do not demonstrate persistent abnormalities in maximal mitochondrial oxidative function, anaerobic glycolysis nor proton clearance as assessed by 31P-MRS. This suggests a likely central component in the pathophysiology of fatigue in GH deficiency.
Declaration of funding
The 1st year of the study was funded by Pfizer Inc and the 2nd year by Merc Serono Inc. However, they were not involved in the design, conduct or analysis of the study.

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P254

Creation of a locus-specific database for *AIP* mutations

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Locus-specific databases (LSDBs) have been recently developed in response to the increasing number of genetic changes reported in the human genome. LSDBs have been created for several genes implicated in endocrine syndromes, for example *MEN1*, *VHL*, *RET*, *GNAS, PRKAR1A* and the *SDH* subunits. Mutations in *AIP* are found in about 20% of familial isolated pituitary adenoma (FIPA) patients.

The aim of this project is to collect all the variants found worldwide in FIPA cohorts accompanied by their clinical information in order to improve our knowledge about this syndrome. We have developed a curated, free-to-use AIP-LSDB, available at http://aip.fipapatients.org/.

The database is registered in Orphanet, the reference portal for rare diseases. *AIP* variants are named according to the locus reference genomic (LRG) 460, a standard reference sequence generated in collaboration with the NCBI and EBI, following Human Genome Variation Society recommendations. Variants submission by other centres is allowed after free registration via a provided clinical description form.

The database is displayed as a graphic view of *AIP*. The number and type of variants in each region (i.e. exons and introns) are reported in a pop-up window while passing with the mouse over the corresponding fragment. By clicking on the region of interest the nucleotide and amino acid sequence appear and all the reported variants are showed underneath as dots. Clicking on each dot enables to see the genetic and clinical details reported in the patients harbouring that variant. A flexible data selection tool is implemented for statistical analysis, but data can also be exported to perform further analyses.

This database will assist clinicians and researchers in the interpretation of *AIP* variants, thus improving genetic counselling and reducing unnecessary testing, and will help to examine the structure–function and the genotype-phenotype correlations, if any, in *AIP* mutated patients.

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P255

High prevalence of pituitary dysfunction following blast traumatic brain injury: results from the UK Blast Injury Outcome Study of Armed Forces Personnel (BIOSAP)

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Background

Pituitary dysfunction is a recognised consequence of traumatic brain injury (TBI) causing significant cognitive, psychological and metabolic impairment. Hormone replacement offers an important therapeutic opportunity. Blast traumatic brain injury (bTBI) from improvised explosive devices (IEDs) is commonly seen in soldiers returning from recent conflicts. We investigated: i) the prevalence and consequences of pituitary dysfunction following moderate-severe bTBI, and ii) whether it is associated with particular patterns of brain injury.

Methods

Nineteen soldiers with moderate-severe bTBI (all male, age: 28.3 years (26.8–32.2), median (interquartile range)), and 39 controls with moderate-severe non-blast TBI (nbTBI) (all male, age: 32.3 (23.1–36.7)), >2 months since injury, underwent full dynamic endocrine assessment. In addition, soldiers had structural brain magnetic resonance imaging (MRI) including diffusion tensor imaging (DTI) and cognitive assessment.

Results

Six of 19 (32.0%) soldiers with bTBI, but only 1 of 39 (2.6%) nbTBI controls, had evidence of pituitary dysfunction ($P=0.038$). Standard MRI failed to show differences between the bTBI subjects with and without pituitary dysfunction. However, DTI measures of white matter structure showed evidence of greater traumatic axonal injury in those bTBI subjects with than without pituitary dysfunction ($P=0.023$) Pituitary dysfunction negatively impacted symptoms, quality of life and cognitive function in soldiers with bTBI. Four out of 19 (21%) soldiers commenced hormone replacement(s) for hypopituitarism.

Conclusions

We reveal a high prevalence of pituitary dysfunction in soldiers suffering moderate-severe bTBI, which was more frequent than after moderate-severe nbTBI. We recommend that all patients with moderate-severe bTBI should routinely have comprehensive assessment of endocrine function.

Declaration of funding

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P256

Correlation of clinical smell test and magnetic resonance imaging of olfactory system in idiopathig hypogonadotropic hypogonadism

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Objectives

i) To measure olfactory bulbs and sulci using dedicated magnetic resonance imaging (MRI) sequences in idiopathic isolated hypogonadotropic hypogonadism (IHH) patients with a well detailed phenotype characterization and ii) to correlate MRI findings with a clinical smell test.

Methods

MRI was performed in 20 patients (all male, aged between 11 and 45 years, mean age of 26) with IHH and olfactory dysfunction was assessed using the smell identification test (UPSIT), a qualitative suprathreshold olfaction test obtained from the Universityof Pennsylvania. Coronal spin echo T2-weighted and volumetric T1-weighted gradient echo sequences were acquired in a 1.5T system. ImageJ software was used to obtain olfactory bulb volumes and olfactory sulcus depths and lengths. Data were analyzed with SPSS 15.0 and the Kappa index was used to evaluate the agreement between the UPSIT and MRI.

Results

The UPSIT revealed normosmia, hyposmia and anosmia in 10 (50%), 4 (20%) and 6 (30%) patients respectively. Fourteen patients (70%) had olfactory abnormalities in the MRI. Commonest abnormality was hypoplasia seen in eight patients (40%). Five patients (25%) had olfactory bulb. One patient had unilateral hypoplasia with normal sense of smell. There was moderate agreement between the MRI quantitative evaluation and the UPSIT (overall $\kappa=0.55$).

Discussion

Olfactory bulb and sulcus aplasia were the most common findings in IHH patients (70%). We objectively demonstrated agreement between MRI findings and the smell test, especially the presence of bulb aplasia and anosmia, confirming the high specificity of MRI findings.

Conclusion

Therefore, our findings help ascertain MRI accuracy in the diagnosis of IHH, differentiating patients with hypogonadotropic hypogonadism with an apparently normal or difficult to evaluate sense of smell.

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P257

Endocrine remission of Cushing's disease after endoscopic trans-sphenoidal surgery: Retrospective review of a single centre experience

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Background

Cushing's disease is caused by corticotroph tumours of the pituitary gland and the standard first-line treatment is trans-sphenoidal surgery. Published data from other centres describes post-operative endocrine remission achieved in 50–90% of cases.

Method

We conducted a retrospective audit of patients who had endoscopic pituitary surgery for suspected or proven Cushing's disease. Data was collected from Jan 2007, when the department commenced endoscopic surgery, until Nov 2012. We

Table 1

	Remission (cortisol <50)	Cortisol (50–150 nmol/l)	Cortisol (>150 nmol/l)
Microadenoma	24	0	4
Macroadenoma	6	2	4
All	30	2	8

analysed patients having their first surgical intervention. For the purpose of this analysis: patients who had surgery followed by a re-exploration during the same admission were treated as having a single surgical intervention. Patients who had undergone previous pituitary surgery were excluded. Post-operative remission was defined as serum cortisol <50 nmol/l within 3 months of the surgical intervention.

Results

We identified 40 cases of suspected or proven Cushing's disease who proceeded to have a first surgical intervention. Pre-operative radiological evaluation revealed 12 had a pituitary macroadenoma (>10 mm), 28 had either microadenoma (<10 mm), normal appearances or a diffusely abnormal intrasellar appearance. The overall post-operative remission rate was 75% (30/40). For microadenoma the remission rate was 86% (24/28). Of the 10 patients not cured by initial surgical intervention: five proceeded to pituitary radiotherapy, three had (or are planned to have) further pituitary surgery, one was cured by unilateral adrenalectomy for ACTH dependant macronodular adrenal hyperplasia and one died of sepsis in the context of severe Cushing's.

Conclusion

The endocrine remission rate for endoscopic transphenoidal surgery for microadenoma is 86%. This is comparable to the highest remission rates reported in the international literature.

Table 1

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P258

Prevalence of familial isolated pituitary adenomas

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While pituitary adenomas (PA) usually occur as a sporadic disease, an increasing number of patients are recognised with a family member also suffering from a PA. If no other syndromic features are present, these families are categorised as FIPA. In published studies, 20% of the FIPA families, 20% of sporadic childhood and 13% of sporadic young-onset (≤30 years) acromegaly patients carry a germline *AIP* mutation. As familial disease is more aggressive, family screening could provide the possibility of early tumour detection and treatment.

Retrospective and prospective audits were performed on our pituitary patient population in terms of family history of PA. Known MEN1 patients were excluded from the analysis.

Retrospective data of 225 patients with acromegaly were analysed; 8 (3.6%) patients from six families were identified with a positive family history. Three were AIP-mutation positive (37.5% of patients with a positive family history, 1.3% of all retrospective patients).

Prospective data gained from a questionnaire of 222 PA patients identified 15 patients (6.8%) with a family history of PA; 64 acromegaly patients were studied (28.3% of the prospective cohort), of which seven patients (10.9%) had a positive family history.

Regarding patients with acromegaly, in the retrospective study 41 (18.2%) and in the prospective study 17 (27%) patients had young-onset disease; 7 (17.1%) young patients in the retrospective and 4 (23.5%) in the prospective study had a family history.

There is a considerable difference in the percent of patients with family history in the retrospective (overall: 3.6%, young-onset: 17.1%) and prospective (overall: 10.9%, young-onset: 23.5%) acromegaly cohort.

These data suggest that almost 7% of an unselected tertiary centre pituitary patient population has a family history of PA. Careful history taking increases the proportion of patients with a family history of acromegaly nearly 3-fold and active inquiry may reveal previously unknown familial connections in all types of PA.

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P259

Short-term (3 months) compared to long-term response to somatostatin analogues in acromegaly

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Objective

To evaluate whether serum GH and IGF1 levels achieved after 3 months treatment with somatostatin analogues (SSA) are concordant with the efficacy of SSA after longer treatment with the same dose.

Patients and methods

From 71 patients with acromegaly treated with SSA in our clinic, in 38 of them (28 women, 10 men, aged 22–62 years) data on serum GH and IGF1 were available at baseline, after 3 months and at the last evaluation on the same SSA dose. Two patients have been evaluated on two different doses of SSA. Optimal response to SSA included random GH ≤2.5 ng/ml and normal age-adjusted IGF1 level.

Results

From 40 evaluations, 18 were on octreotide LAR (9 on 20 mg, 9 on 30 mg/month) and 22 were on lanreotide SR (15 on 60 mg/month, 7 in 90 or 120 mg/month). Three evaluations were before neurosurgery and 25 patients had pituitary radiotherapy before SSA. Mean duration of treatment was 14±9.5 months (5–44) .

Normal random GH was recorded at 3 months in 21/39 patients (53.8%) and at the last evaluation in 24/39 patients (61%). Concordant values between the two points of evaluation were found in 28/39 patients (71.8%). Mean±S.D. GH values were 6.1±9.8 and 5.7±11.9 ng/ml respectively (P=NS). Normal serum IGF1 was recorded at 3 months in 20/35 patients (57.1%) and at the last evaluation in 17/35 patients (48.5%), concordant values in 31/35 patients (88.5%). Mean IGF1 levels (×ULN) were 1.3±0.82 and 1.3±0.84 respectively. Normal values at 3 months and elevated at the last evaluation were found for GH in 4/39 patients (10%) and for IGF1 in 4/35 patients (11.4%, 3 of them up to 1.3×ULN). Normalization only at the last evaluation was recorded in 7/39 patients (18%) for GH.

Conclusion

In patients with acromegaly the response to somatostatin analogues evaluated at 3 months was concordant with the response after longer treatment with the same dose in about 72% of patients for GH and 89% for IGF1. When discordances between normal IGF1 and elevated random GH occur at 3 months, we suggest re-evaluation on the same SSA dose.

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P260

Incidental pituitary haemorrhage is common in prolactin-secreting macroadenoma especially in women

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Background

Incidental pituitary haemorrhage, not associated with pituitary apoplexy, is a common clinical and radiological finding. Little information exists on the clinical behaviour of incidental haemorrhage with most reports describing surgically treated macroprolactinoma and non-functioning adenoma, and there are few data in a clinic prolactinoma population.

Aims

To characterise the prevalence, natural history and risk factors associated with pituitary haemorrhage in a large clinic prolactinoma population.

Method

A retrospective case-note analysis of 368 patients with prolactinoma presenting to Guy's and St Thomas' Hospitals between 2000 and 2008. Presence of haemorrhage was noted on magnetic resonance imaging (MRI).

Results

Pituitary haemorrhage was found in 25 patients, giving an overall prevalence of 6.8%, and was significantly higher in macroprolactinoma (20.3%) than in microprolactinoma (3.1%) (P<0.0001). Three patients had classical pituitary apoplexy. The majority of patients in the haemorrhage group had macroprolactinomas (16/25 (64%)) and the majority were female (22/25 (88%)). The proportion of females with macroprolactinoma was also higher in the haemorrhage group (14/16 macroprolactinomas (87.5%)) than in the non-haemorrhage group (36/63 macroprolactinomas (57.1%)P=0.02).The majority of patients were treated conservatively (92%) with 87% of patients having

complete resolution of their haemorrhage within 26.6±5.2 (mean±S.E.M.) months. Anticoagulant therapy, diabetes, hypertension and different types of dopamine agonist therapy were not associated with pituitary haemorrhage. After adjustment for confounders, the presence of macroprolactinoma (odds ratio 9.00 95% CI 3.79–23.88 $P<0.001$) and being female (odds ratio 8.03 (95% CI 1.22–52.95) were independently associated with haemorrhage.
Conclusion
These data suggest that haemorrhage is common in macroprolactinoma where one in five develop haemorrhage, but is also present in microprolactinoma. The vast majority resolved spontaneously with medical treatment. We also present novel data showing a strong female preponderance, suggesting that women, particularly with macroprolactinoma, were more likely to develop haemorrhage.

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P261

Ophtalmological, endocrine, and neurological complications in giant male prolactinomas.

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Introduction
Prolactinomas are more invasive in males. Giant ones (height ≥4 cm) are relatively rare in literature.
Our aim is to analyze their frequency, their radiological aspects, and their neurological, endocrine and ophthalmological complications.
Methods
All of them had clinical exam, hormonal, ophthalmological, and radiological assessment based on cerebral MRI. Mixed adenomas were excluded. Positive diagnosis was based on clinical presentation, high prolactin concentration, positive response to dopamine agonists±immunohistochemy study.
Results
Among 154 male prolactinomas seen in our department between 1984–2012 we have observed 44 giant tumors (28.5%). Mean age=36 years, 38.3% were under 30. Mean tumor height=53.95 (40–97) mm and mean volume=66.2 mm^3 (15.5–184). Mean prolactin =15 715 ng/ml ($n<15$). Solid and kystic aspect, with or without calcifications, mimicking craniopharyngiomas was observed in 28.5%. Cavernous sinuses were invaded in all except two. Other invasions were: posterior=69.4%, anterior =58.3%, and frontal=5.5%. For endocrine complications we observed gonadic deficit in 98.4%. Thyreotro and corticotrop insufficiencies were seen in respectively 34%, and 32%. 47% had multiple deficits. Posterior pituitary deficit was observed in 2.2%. Ophthalmological complications were: Optic atrophy in 46%, ptosis=8%, diplopia±strabismus =5.4%. For neurological abnormalities we observed memory loss and/or unconsciousness=24%, epilepsy=15.5%, compressive hydrocephaly=8% and frontal syndrome=5%.
Conclusion
In this study concerning giant male prolactinomas, optic atrophy is the most common abnormality. Severe and life threatening neurological troubles are very frequent too as they were observed in nearly 40%. But, multi pituitary deficits and compressive hydrocephaly are relatively rare, which argues for a low progression.

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P262

Endocrine and radiological abnormalities in empty sella syndrome

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Background
Primary or idiopathic empty sella syndrome (ESS) is the herniation of the meninges through an incompetent diaphragma sellae into the sella turcica which pushes the pituitary gland aside so giving the appearance of an empty sella. Secondary ESS is caused by damage to pituitary tissue which results in an empty sella turcica. There is significant lack of agreement in the literature regarding the number of patients with empty sella syndrome (ESS) who suffer from pituitary dysfunction; some papers report that pituitary function is usually preserved while others state that it is not.
Aims
The aim of this study was to determine the proportion of patients with ESS who had endocrine abnormalities on presentation and to define radiological features to differentiate between primary and secondary ESS.
Methods
In this retrospective study, records of all patients diagnosed with an 'empty sella turcica' at since 1990 were reviewed. 26 patients satisfied these criteria and their MRI scans were re-evaluated blind by a neuroradiologist to confirm a diagnosis of primary or secondary ESS. Results of baseline endocrine testing and MRI scans were recorded. Information on demographics, presenting complaint and blood pressure were also collected.
Results
65% ($n=17$) of patients diagnosed with ESS showed evidence of pituitary dysfunction. The most common endocrine abnormality was GH deficiency which affected 54% ($n=14$) followed by secondary hypothyroidism in 9 patients with low levels of TSH and T_4. Headache (39%) and fatigue (27%) were the most common presenting complaints.
Conclusion
ESS is a heterogeneous condition with varied, nonspecific symptoms and high rate of endocrine dysfunction. Endocrine function should be assessed in all patients with this diagnosis.

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P263

^{11}C-methionine PET–CT co-registered with volume MRI identifies residual functioning tumour in acromegaly

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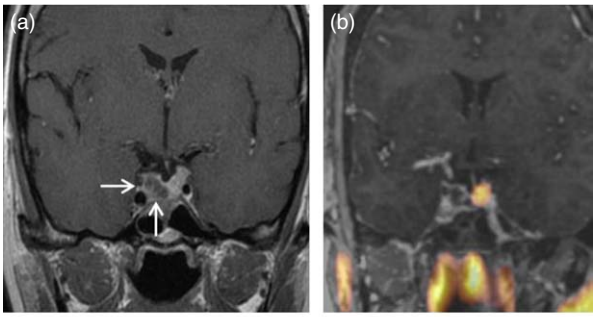
Although MRI remains the investigation of choice for pituitary imaging, it does not reliably identify all secretory microadenomas, and cannot always discriminate residual tumour from post-surgical change following hypophysectomy. We hypothesised that i) imaging with the PET ligand ^{11}C-methionine, which is taken up at sites of peptide/protein synthesis, would permit more reliable identification of functioning pituitary adenoma, and ii) co-registration of PET–CT with volume MRI (MetPETCT-MRI) would yield more accurate anatomical localisation of ^{11}C-methionine uptake.

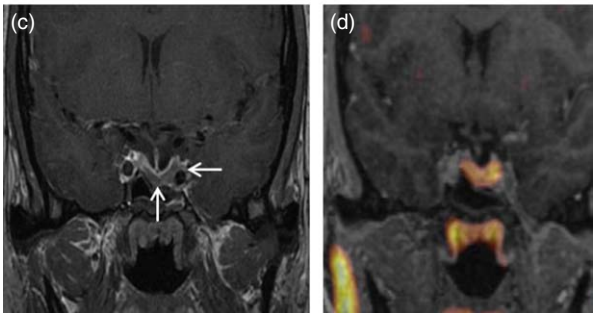
We studied subjects with acromegaly in whom MRI had identified possible residual tumour following transsphenoidal surgery (TSS). We deliberately chose patients with acromegaly to allow correlation of radiological findings with clinical and biochemical markers of disease activity.

In three patients with suspected residual tumour on post-operative MRI (Fig. 1a *arrows*), but in whom clinical and biochemical assessment confirmed remission, MetPETCT-MRI showed no corresponding pathological tracer uptake (Fig. 1b). In contrast, in three patients with persistent active acromegaly following surgery, MetPETCT-MRI demonstrated tracer uptake at the site of suspected residual tumour (Fig. 1c,d), which was confirmed at repeat TSS in one patient (the other two are awaiting surgery). In another patient, who elected to be treated with primary medical therapy, but in whom adequate control was not achieved despite somatostatin analogue and GH antagonist therapy, MRI revealed only a thin rim of tissue lining an enlarged pituitary fossa. However, MetPETCT-MRI demonstrated focal tracer uptake, which was confirmed at subsequent surgery to be due to a strongly positive GH-staining somatotroph adenoma

Subject 1 – in remission



Subject 2 – residua active acromegaly



Our preliminary findings suggest that MetPETCT-MRI is a useful adjunct for identifying residual pituitary tumour when MRI appearances are inconclusive. As peptide/protein synthesis is a common property of all pituitary tumours (including so-called non-functioning pituitary adenomas) it is likely that MetPETCT-MRI will find application in all tumour subtypes.

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P264

A retrospective cohort study of patients with hyperprolactinaemia

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Hyperprolactinaemia is the most common endocrine disorder of the hypothalamic-pituitary axis and represents a significant referral volume to secondary care. It is therefore necessary to employ a timely, structured management pathway.

This retrospective cohort study at University Hospitals Birmingham, included 450 patients with serum hyperprolactinaemia, measured between 2011 and 2012. Patients with a known hyperprolactinaemia were excluded. 71 patients remained for subsequent analysis. Data presented does not include patients with hyperprolactinaemia measured in the community whose prolactins had normalised at time of review.

Of the 71 patients identified 62.0% were female. The most common presenting complaint was either oligomenorrhoea or amenorrhoea (29.6%). Other significant presenting complaints included: galactorrhoea (15.5%); headache (14.1%); visual field defects (8.5%) and erectile dysfunction (7.0%). Patients with persistent hyperprolactinaemia underwent dedicated MRI pituitary.

The predominant aetiology was microprolactinoma (18.3%) (mean prolactin 2118.9) and non-functioning pituitary adenoma (15.5%) (mean prolactin 810.5). Of those with microprolactinoma the majority were managed with cabergoline (50%). Drug induced hyperprolactinaemia accounted for 14.8% of patients (mean prolactin 1352.5); mostly due to risperidone (66.7%). Management of these patients proved difficult given the persistent requirement for the causative drug.

In the secondary care setting at University Hospitals Birmingham the investigation and management of both incidentally and rationally identified hyperprolactinaemia represents a significant service burden. five of the ten patients thought to have drug induced hyperprolactinaemia underwent MRI pituitary and 100% of these scans were normal. It may therefore be worth rationalising the use of imaging in such cases and electing for a change in medication and repeat measurement of prolactin. The exclusion of patients whose community measured hyperprolactinaema had normalised limits cohort number. It also suggests that in cases of incidentally found hyperprolactinaemia with a borderline result repeating this result in the community may prevent referral.

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P265

Examining the distribution of abdominal fat in GH deficiency using magnetic resonance imaging

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Background

Adults with GH deficiency (GHD) have altered body composition with an increase in abdominal fat when compared with healthy matched controls. However, most studies have not compared GHD adults with GHD adults on GH replacement.

Abdominal fat is composed of subcutaneous abdominal tissue (SAT) and visceral abdominal tissue (VAT). Increased VAT is associated with poor metabolic outcomes. Magnetic Resonance Imaging (MRI) is a reliable and reproducible means of quantifying abdominal fat distribution. Our aim was to assess and compare VAT and SAT compartments in untreated GHD adults, treated GHD adults and matched healthy controls.

Methods

Eighteen untreated GHD adults, 17 treated GHD adults and 19 age and sex matched healthy volunteers were recruited. Fifteen patients had combined pituitary hormone deficiency in both the untreated and treated GHD groups. The remaining had isolated GHD. All patients underwent anthropometric assessment, bio impedance analysis and MR Imaging of their abdomen at the level of L4/L5. Minitab v16 was used for statistical analysis.

Results

Matched healthy controls had lower body fat % ($P=0.046$), total abdominal fat ($P=0.021$), SAT ($P=0.031$) and VAT ($P=0.028$) when compared to GH deficient patients off and on GH replacement. GH replacement was not associated with changes in body fat %, total abdominal fat, VAT or SAT. There was no difference in fat distribution (VAT/SAT ratio) between the three groups ($P=0.47$).

Conclusions
There were no major alterations in body fat distribution in untreated GHD adults when compared with treated GHD adults. However, both treated and untreated GHD adults have increased body fat % and abdominal fat when compared to healthy controls. This is likely to reflect the causes and consequences of hypopituitarism rather than the effects of GH deficiency in isolation. We conclude that both treated and untreated GHD patients have increased fat mass but the distribution of this fat is no different to control subjects.
Declaration of funding
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P266

Long-term results after treatment of craniopharyngioma: experience with 46 adult patients

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Background:
Craniopharyngioma is a rare, mostly benign tumor of the central nervous system, generally associated with important morbidity.
Aim:
To study the clinical characteristics and treatment outcome in adult patients.
Methods:
Adult patients diagnosed with craniopharyngioma between 1980 and 2012, followed-up in the Pituitary and Neuroendocrine Department of the 'C.I.Parhon' National Institute of Endocrinology in Bucharest were retrospectively evaluated. We studied the presenting symptoms, complications at diagnosis, type of treatment, surgical complications, rate of tumor resection, endocrinological and visual outcome.
Results:
A total of 46 patients (18 females, 28 males aged between 18 and 72 years, median 35.4 years) with a mean follow-up of 7.33 years (1–41 years) were included. The presenting symptoms were mostly headache (86.95%), visual impairment (78.26%), symptoms of hypopituitarism (30.42%), diabetes insipidus (DI) (8.69%), hydrocephalus (15.21%); the median time to diagnosis was 12 months. All tumors had sellar and suprasellar component, the mean craniocaudal diameter was 2.58 cm (range 1 – 5.6 cm). All patients were operated (69.56% – transfrontal approach, 26.08% transsphenoidal, 4.34% – frontotemporal); in most cases repeated surgery was necessary due to recurrence or remnant growth. six cases received adjuvant radiotherapy. In only 13 cases (28.26%) gross-total removal (GTR) was achieved (3 subsequently recurred), in 71.73% of cases a tumor remnant (diameter between 0.4 and 5 cm) was present. 32.6% developed permanent DI. Visual field improved in 15.21% (normalized in four cases), worsened in 36.95% and remained stable in 45.65%. Two hypopituitary cases normalised, in all others (89.13%) hypopituitarism remained or worsened with treatment. Only 56.52% of cases could return to their usual activity. Weight gain was significant: 38% of normal or overweight cases became obese.
Conclusions:
Craniopharyngiomas are often associated with an ominous prognosis and significant morbidity. Surgery remains the treatment of choice but total resection can be achieved in a minority of patients in our center.
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P267

Syndrome of inappropriate antidiuretic hormone secretion and treatment with tolvaptan: a case series

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Background
Hyponatraemia is the most common electrolyte abnormality, encountered in up to 30% of inpatients. Plasma sodium levels <125 mmol/l are classed as severe and can be associated with drowsiness, confusion, ataxia and personality change. In mild hyponatraemia, the patient is usually asymptomatic, although studies have shown chronic low sodium of any level may be associated with increased mortality and longer hospital stays. Vasopressin receptor antagonist (tolvaptan) are indicated to treat hyponatraemia in patients with SIADH not responding to fluid restriction. In this case series we present our experience with the use of Tolvaptan. Methods:
A retrospective study of all patients treated for SIADH with Tolvaptan. Patient demographics and biochemistry were obtained from the case records. Results: Nine patients were identified who were treated with tolvaptan; and included five males, mean age 74.4 ± 8.5 years. Cause of SIADH were: pituitary tumor (1), Lung cancer (3), Drug induced (1), stroke (2), idiopathic (2). Mean admission serum sodium (S Na) was 120 ± 9 mmol/l. After fluid restriction mean S Na was 122.9 ± 4.2 mmol/l. Following commencement of tolvaptan (15 mg daily) serum sodium at 24, 48–72 h and 1, 2 and 4 weeks were: 128.8 ± 4.3, 133.1 ± 2.8, 133.2 ± 3.8, 133.1 ± 5.5, 133.2 ± 4.7 mmol/l respectively. Mean (range) duration of tolvaptan therapy was 81 (5–180) days. Five patients had S Na >130 in 24 h, and all patients had S Na >130 within 72 h. None of the patients had side effects to tolvaptan. One patient had a greater than recommended increase in serum Na (>12 mmol/l) in 24 h. Conclusion: Patients treated with tolvaptan had rapid improvement in serum sodium levels. Use of tolvaptan was without any adverse events to the treatment.
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P268

Hyponatraemia assessment and outcomes in acute medically ill patients

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Background
Hyponatraemia is the most common electrolyte abnormality, encountered in up to 30% of inpatients. Inappropriate management can have serious implications for patients; including demyelinating disease, coma, and death.
Methods
Patients ($n=122$) admitted to the medical admission unit of a district general hospital with a serum sodium (Na) <130 were selected for the study. All details including patient demographics, blood biochemistry, date of admission and date of death were taken from the case notes and hospital computerised system. Details on assessment of hyponatraemia including thyroid, adrenal and renal function were also recorded.
Results
Mean age was $70.4+18.1$ years; 48 males. Mean serum Na on admission was $125.8+4.1$ mmol/l. Of the 122 patients, 38 died (31.1%) in hospital. Patients who died were older (66.7 ± 18.9 vs 72.3 ± 15.3; $P=0.054$) Admission serum Na and plasma glucose in survivors vs died was 127.2 ± 3.9 vs 124.2 ± 4.7 mmol/l ($P<0.001$); and 7.0 ± 3.0 vs 5.4 ± 2.2 mmol/l ($P=0.02$) respectively. Patients with admission serum Na <125 in the survivors vs died was 32.1 vs 66.7%. Patients investigated for hyponatraemia were as follows: serum cortisol ($n=6$), plasma osmolality ($n=9$), urine osmolality ($n=9$), short synacthen test ($n=0$) ,urine sodium ($n=3$), thyroid function tests ($n=19$). Conclusions: Patients admitted with acute medical conditions with severe hyponatraemia have a high mortality and those with lower serum sodium have greater risk of death. Patients were also inadequately worked up for assessment of cause of the hyponatraemia and further education of medical specialists is urgently required to improve management and outcome. Also lower admission plasma glucose was associated with higher mortality.
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P269

Inside acromegaly: a pilot study for recruiting focus groups using social media

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Background
The exponential growth of social media over the last decade has provided new, diverse and accessible ways in which people can share and receive information. Its rapid rise has attracted researchers and professionals of various disciplines, aiming to capitalise on this relatively new phenomenon. In this qualitative study we utilised social media to conduct research on psychosocial aspect of acromegaly. Facebook currently has 845 million users. Acromegaly, with a prevalence of approximately 60/million, was used as an exemplar of a relatively

rare disease associated with adverse physical and psychological effects.
Method
A Facebook account was created and 10 international acromegaly Facebook groups contacted. In-depth semi-structured interviews were conducted using Facebook chat, messenger or Skype, and a focus group established on Facebook. Four groups responded and in addition to nine individuals who participated in a focus group, eigth agreed to an in-depth semi-structured interview of which five were completed.
Results
Analysis of the data revealed various emerging themes each classed under one of three super-ordinate themes: i) psychological impact ii) social impact (3) implications for practise. Changes in physical appearance and low mood associated with the acquisition of disfigurement later in life were the predominant factors responsible for depression, social withdrawal and adverse effects on occupation and relationships.
Conclusion
Using social media to recruit focus groups is a very rapid and economical way to gain potentially important insights into the social impact of rare diseases such as acromegaly.

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P270

Pituitary apoplexy: a case series

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Pituitary apoplexy whether due to haemorrhage or infarction remains a rare endocrine diagnosis. Recent UK guidelines have emphasised the lack of published evidence in the management of this condition. We present our experience of 12 current cases (nine males, three females).
Eleven cases were managed conservatively (91.6%), one patient required urgent pituitary surgery. None of the above cases required pituitary radiotherapy.
Presenting symptoms were headache and meningism (75%) with ocular palsy and visual field defects in three (25%) of patients. Interestingly, 25% of patients presented with non-specific symptoms of fatigue and no headache. Clinical suspicion of Apoplexy was high in 58% of cases.
In terms of initial endocrine deficit, nine (75%) patients required steroid replacement ab initio (2 patients were successfully weaned-off, but two more had to be started on steroid replacement), consequently 75% continue on steroid replacement. 25% of patients required Levothyroxine from diagnosis with 58% currently on thyroid hormone replacement. One patient was hypogonadal at diagnosis and six more (58%) are currently on Testosterone. One patient developed partial Diabetes Insipidus and is on Desmopressin and three patients are on growth hormone replacement. Three patients so far are currently on no hormone replacement therapy. Two of these patients are on Dopamine agonist therapy for their macroprolactinomas. All other tumours are currently believed to be non-functional.
In all cases there was no evidence of tumour re-growth. Tumour shrinkage was on average 34.8% (tumour width) and 25.1% (tumour height) over an average of 3.4 years.
In conclusion, although the majority of cases have presented with the classical picture of sudden onset of headache and meningism, this was not universal. Endocrinologists do need to be aware that apoplexy can present with non-specific, non-classical symptoms.
A conservative management approach in certain clinical circumstances seems to provide satisfactory outcomes.

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P271

An unusual cause of testosterone deficiency

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36-year-old gentleman referred by his GP with poor libido and erectile dysfunction associated with hypogonadotrophic hypogonadism. 0900 h testosterone 0.3 nmol/l (10–35), LH <0.2 IU/l, FSH 0.1 IU/l, prolactin 71 mIU/l (50–500).
Symptoms started at time of break up of his marriage in 2011. No other symptoms of hypogonadism or of pituitary disease. Previously fit and well. Teetotal. On no medication. Patient an avid fitness fanatic. He had never knowingly used anabolic steroids. However, he had taken a 'nutritional supplement' called T-Bullets (purchased from a sports nutrition shop to improve his gym performance) for 20 days until just before GP's blood test. Examination revealed a well virilised gentleman with no signs of hypogonadism.
Further investigations
0900 h cortisol 457 nmol/l (138–690); IGF1 331 μg/l (75–344); FT_4 11 pmol/l (5.6–21); Normal U&E, FBC and LFT. MRI pituitary and hypothalamus within normal limits
6 months after stopping T-Bullet, testosterone level gradually returning to normal. 04/10/12 – 6.9 nmol/l, 29/10/12 – 10.2 nmol/l
Discussion
T-Bullets are marketed as a 'nutritional supplement' by the makers. They are easily available online and can be purchased from some sports nutrition shops. The active ingredient is: '2a, 17a-dimethy-5a-androst-3-one-17b-ol 13-ethyl-3-methoxy-gona-2,5,(10)-dien-17-one' which Martindale: the complete drug reference lists as an anabolic steroid (related to testosterone)
Conclusion:
Anabolic steroids were first artificially synthesized in the 1930s. The misuse of anabolic steroid drugs to enhance physique, body strength and athletic performance is well-known. Use of anabolic steroids can result in hypogonadotrophic hypogonadism as a result of suppression of the hypothalamo-pituitary–gonadal axis (HPG).
The unwitting consumption of an anabolic steroid should be considered in any patient presenting with hypogonadotrophic hypogonadism who has been taking a 'nutritional supplement'.
The unwitting consumption of an anabolic steroid should be considered in any patient presenting with hypogonadotrophic hypogonadism who has been taking a 'nutritional supplement'.

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P272

Rare case of round blue cell pituitary tumour with probable hypothalamic involvement

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Introduction
We submit a rare presentation of round blue cell pituitary tumour complicated by cranial diabetes insipidus following transphenoidal surgery.
Case history
47-year-old lady with severe headache was diagnosed with a 10 mm non functioning pituitary macro adenoma. Initial plan for conservative management was revised as she developed sixth cranial nerve palsy, bi temporal hemianopia and rapidly enlarging pituitary tumour to 20×18×19 mm. Urgent Transphenoidal pituitary decompression was performed. However the headache got worse and the cranial nerve palsy did not resolve. As the vision significantly deteriorated, she underwent a repeat surgery in 2 weeks. Pituitary tumour was found to be of fibrous consistency and the histology confirmed poorly differentiated round blue cell tumour with mitotic index 50%, positive for CD99 and CD56, cytogenetically unclassified.
Management and discussion
On 1st post-operative day, she developed polyuria and polydipsia, diagnosed with cranial DI and commenced on DDAVP. Next day she developed generalised seizure caused by rapidly developing hyponatraemia, managed with strict fluid balance and a fluid restriction of 1.5l daily with dynamic management DDAVP dose. Chemotherapy with Vincristine, Doxorubicine, Cyclophosphomide and high dose of Dexamethasone started. She developed steroid-induced diabetes mellitus, managed with Glargine. She became very thirsty despite normal serum sodium and urine osmolality raising the possibility of hypothalamic thirst centre being affected by the aggressive pituitary tumour. Patient had some improvement in her vision after two cycles of chemotherapy and it was planned to complete 4 cycles followed by high dose radical adjuvant radiotherapy with an optic chiasm sparing regime. Overall prognosis remains poor.
Conclusion
We presented this case to illustrate a rare, aggressive pituitary malignancy which possibly has invaded the hypothalamus causing disruption to the thirst mechanism in addition to cranial diabetes insipidus.

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P273

Pituitary abscess: a rare cause of pituitary mass lesion

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Pituitary abscess is very rare accounting for 0.2 – 0.6% of all pituitary lesions. Only around 210 case reports have been described so far. We report a case of pituitary abscess presented as pituitary mass lesion with hypopituitarism.
A 51 year old man was admitted with headache and found to have severe hyponatraemia. Past medical history included paranoid schizophrenia. Clinical examination was normal.
Relevant investigations: sodium 110 mmol/l, 0900 h cortisol 21 nmol/l, free T_4 5.1 pmol/l, TSH 0.34 mIU/l, LH 0.8 IU/l, FSH 3.4 IU/l, testosterone 0.6 nmol/l, prolactin 139 mU/l.
Pituitary MR revealed a macroadenoma measuring 20 mm and abutting the optic chiasm. Glucagon stimulation test confirmed secondary hypoadrenalism. Visual field tests showed bilateral superior upper quadrantanopia.
He was started on hydrocortisone, thyroxine and testosterone replacement for the hypoptuitarism. He was referred to the neurosurgical team. He underwent trans sphenoidal surgery. Creamy soft material drained. Histology revealed fluid with acute and chronic inflammatory cells consistent with abscess. He has received antibiotics. Post operatively he recovered well and headaches improved.
Conclusions:
Pituitary abscess usually occurs in pre-existing pituitary lesion. Predisposing factors usually include focus of parasellar infection. Usual presenting features include headache, visual defects, hypopituitarism, pyrexia and meningitis. MRI with contrast may show peripheral enhancement. Treatment is surgical drainage, antibiotics and hormone replacement.
Pituitary abscess is very rare, a potential cause of pituitary mass. Clinical diagnosis should be suspected with symptoms and signs of pituitary mass and infection. It's often radilogically indistinguishable from other pituitary lesions. Correct diagnosis is difficult before the surgery.

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P274

Hypopituitarism presenting with features of stiff person syndrome

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Introduction
Stiff person syndrome (SPS) is a progressive neurological disorder characterized by fluctuating stiffness and rigidity in both axial and limb muscles. Stiff leg syndrome, an SPS variant mainly affecting limb muscles, is emerging as a distinct identity. The cause of SPS is unknown but an autoimmune pathogenesis is suspected. There are a few reported cases of hypopituitarism presenting with features suggestive of SPS.
Case
A 66-year-old woman presented to the neurologists with a 12-month history of gradually worsening back pain, leg spasms resulting in muscle stiffness and difficulty mobilising. Physical examination showed lower limb rigidity but normal sensorimotor function and reflexes.
MR brain scan showed minimal pituitary enlargement without any focal abnormality. Endocrine assessment revealed GH, TSH, gonadotropin and partial ACTH deficiency. Electromyography and anti-GAD antibodies were negative, but her presentation was felt to be consistent with SPS. Despite a favourable initial response to GABA-enhancing drugs, a lasting clinical remission was only achieved with hydrocortisone replacement therapy.
Discussion
We report this unusual case of hypopituitarism presenting in a patient with clinical features suggestive of SPS. Appropriate endocrine evaluation and pituitary hormone replacement may alleviate the significant morbidity associated with this condition.

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P275

Snake bite and hypopituitarism: ignorance or incompetence?

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Hypopituitarism as a consequence of snake bite is rare. However, there are a few case reports from India and adjacent South-East Asian countries. We present a case of hypopituitarism secondary to snake bite where the diagnosis was significantly delayed. A 42-year-old gentleman presented as out-patient with a 10 years history of generalized weakness, lethargy, coarse facial features, reduced libido, reduced socialization and depression. He had an acute illness following a snake bite 15 years ago. He was also diagnosed with hypothyroidism 11 years ago. The snake bite was associated with altered sensorium and acute renal failure requiring dialysis for several days. He was managed by general physicians who diagnosed hypothyroidism and commenced levothyroxine. His symptoms persisted, for which his levothyroxine dosage was reorganized many times and multivitamins prescribed. Review of the original TFTs were consistent with secondary hypothyroidism, which had been overlooked by his treating physicians. Further investigations revealed fT_4 8.6 (9.0–20.0) pmol/l, TT_3 1.32 (0.6–1.80) ng/ml, TSH 0.00 (0.35–5.5) μIU/ml, 8am cortisol 0.00 (4.30–22.40) mg/dl, ACTH 6.10 (7.2–63.3) pg/ml, LH $<$0.10 (1.1–7.0) mIU/ml, FSH $<$0.10 (1.7–12) mIU/ml, GH $<$0.05 (0.00–4.00) ng/ml, IGF1 $<$25 (101.00–267.00) ng/ml, IGFBP3 1.01 (3.30–6.60) μg/ml, and testosterone $<$0.10 (3.0–10.6) ng/ml. Pituitary MRI Scan revealed an 'Empty Sella'. Hypopituitarism was diagnosed and he was immediately commenced on Hydrocortisone 10-5-5 mgs. He was subsequently started on Testosterone gel, later changing to testosterone depot injections 10 weekly. His symptoms improved significantly over a few weeks.
Discussion
The type of snake bitten determines the symptoms and signs of envenomation. Viper bites are venomous and cause altered sensorium, coagulopathy, internal bleeding, hypotension, tachycardia, renal and respiratory failure. Our patient's symptoms were consistent with a viper bite. His hypopituitarism and secondary hypothyroidism remained undiagnosed leading to physical and mental suffering for more than 10 years. We suspect that the patient developed pituitary apoplexy and chronic hypopituitarism resulting from the snake bite. Endocrinologists should be aware of the possibility of hypopituitarism in patients who suffer a snake bite.

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P276

TSH-secreting pituitary adenoma identified in pregnancy: management of an unusual case

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A 35-year-old woman was referred with biochemical hyperthyroidism (T_4 30 pmol/l; T_3 7 pmol/l) without TSH suppression (5.4 mIU/l). She was 7 weeks pregnant following natural conception, and reported no symptoms of hyperthyroidism. Migraines had been a feature in very early pregnancy, but her medical history was otherwise unremarkable and there was no significant family history. Examination findings were entirely normal.
Investigations confirmed negative thyroid heterophile antibodies. Interpretation of usual investigations for a TSH-secreting pituitary adenoma was complicated by pregnancy (elevated α-subunit $>$24 μg/l, prolactin 722 mIU/l and SHBG 209 nmol/l; LH and FSH fully suppressed). Pituitary MRI showed asymmetrical enlargement (16$\times$11 mm) considered to be disproportionate to the gestational period. Mutational analysis of the thyroid hormone receptor gene was negative (although 15% of cases have no detectable mutations).
As thyroid hormones were only mildly elevated, surveillance was commenced with regular thyroid function tests and trimesteral visual field assessment. There were no fetal or maternal complications encountered and she had a normal delivery at term. *Post-partum* investigations show persistent α-subunit elevation (4 μg/l), but the rest of her hormone profile including prolactin has normalised. We are currently planning future management.
There are only three reports of management of TSH-secreting pituitary adenoma in pregnancy, all identified before conception. Issues raised here are i) the difficulty distinguishing thyroid resistance syndrome from pituitary driven disease in pregnancy given the potential for false negative results and changes in hormone profiles; ii) the challenges identifying the appropriate time to commence treatment given the elevated risk of miscarriage and fetal morbidity in association with maternal hyperthyroidism; iii) concerns for potential complications of accelerated hyperthyroidism (due to the βHCG surge) or visual disturbance (due to pituitary enlargement); and iv) identifying the appropriate treatment to commence should it be required.

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P277

A rising TSH in a patient with known TSHoma does not necessarily indicate recurrence

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In 2003, a 60-year-old man presented to our unit non-specifically unwell. Thyroid function tests (TFTs) demonstrated an elevated fT_4 of 50 pmol/l (reference range 10–22 pmol/l)), T_3 8.8 pmol/l (reference range 3.1–6.8 pmol/l) and TSH of 10.3 mU/l (reference range 0.5–4.5 mU/l)). Following appropriate investigations, a TSHoma was confirmed. MRI of the Pituitary confirmed the finding of a macroadenoma and the patient underwent pituitary decompression with subsequent stereotactic radio-surgery in 2004. Interval MRI Scans remained stable with no suggestion of change in residual pituitary tissue.

Last year, after having felt well for a number of years, he gave a 1-year history of feeling generally tired and lethargic, with weight gain, and a new diagnosis of depression. Thyroid function showed a rising TSH. Repeat free T_4 levels were within the reference range. Repeat MRI did not identify any change in size of the remaining pituitary tissue.

Repeat testing of his TFTs demonstrated a trending increase in TSH with a falling T_4 level (albeit still within the reference range). Thyroid peroxidase antibodies were requested which were strongly positive (2827 Iu/ml, reference range <100 Iu/ml), suggesting likely, co-existent thyroiditis.

Treatment with levothyroxine has been considered but withheld currently as the free T_4 remains in the normal range. He is under active monitoring and there has been a return to his pre-existing pattern of TFTs indicating a likely diagnosis of a transient thyroiditis.

This case highlights the importance of maintaining vigilance and an open mind for co-existent common pathologies alongside rare ones in the same organ system and that not all rising TSH values indicate relapse of TSHoma.

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P278

Unusual presentation of acromegaly and functioning pituitary gonadotrophinoma (FSHoma)

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Background

Gonadotroph adenomas are the most common non-functioning pituitary adenomas in adult[1, 2]. However, functioning gonadotrophinomas are rare. Here, we describe an unusual presentation of concurrent acromegaly and functioning FSHoma (FSH-secreting pituitary adenoma).

A 39-year-old man presented with a vague visual disturbance to the optometrist and a bitemporal hemianopia was detected.

Further questioning elicited increase in shoe size with no significant history of sweating, headache and change in hand size.

Physical examination was notable for features of acromegaly (prominent eyebrows, prognathism, large hands and feet) and bilateral testicular enlargement.

Endocrine profiling confirmed acromegaly on OGTT- oral glucose tolerance test (non-suppressed nadir GH level of 1.2 μg/l with basal GH level of 1.5 μg/l). IGF 1 level was elevated at 64 (9.5–45) nmol/l. There was significantly elevated FSH level of 107.2 (1–10.1) U/l with LH 1.2 (1.5–6.3) U/l and testosterone 9.3 (8–29) nmol/l. The rest of pituitary profiling was normal: Prolactin 370 (45–375) mU/l, TSH 0.65 (0.35–5.5) mU/l, FT_4 12.3 (10–19.8) pmol/l, normal short synacthen test.

US testes confirmed bilateral testicular enlargement: left testis measuring 46 cc and right testis measuring 50 c with no neoplastic changes.

MRI pituitary demonstrated a large pituitary macroadenoma 3.5×2.8 cm, with the expansion of pituitary fossa, compressing optic chaism and extending into left cavernous sinus.

Subsequently transsphenoidal hypophysectomy was performed. Histology confirmed chromophob pituitary adenoma. Immunohistochemistry showed FSH-immunoreactive adenoma with negative GH staining.

Literature analysis revealed very rare synchronous presentation of acromegaly and FSH-secreting pituitary adenoma[3]. Concurrent GH and prolactin secretion is more common.

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P279

Pituitary apoplexy and aortic dissection

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Introduction

Pituitary apoplexy is a rare but life threatening clinical syndrome characterised by acute neuro-ophthalmological features caused by haemorrhage and/or infarction of the pituitary gland. Although many precipitating factors are known, most apoplectic episodes occur spontaneously.

Case

A 59-year-old gentleman presented in 2005 with a 12-month history of bitemporal hemianopia. An MR scan revealed a pituitary macroadenoma. When he was reviewed in the pituitary clinic repeat assessment showed improvement of the visual field defect. His baseline pituitary hormonal profile was normal. In the absence of any signs of optic chiasmal compression and given that he had significant co-morbidity by way of CCF, a watch and wait policy was adopted. He remained clinically and radiologically stable for the next 5 years.

In 2010, he was admitted to a neighbouring hospital with acute aortic dissection. This was managed conservatively and was followed by a protracted period of rehabilitation. During his recovery he was found to have a bitemporal hemianopia and was therefore assessed in the pituitary clinic. His visual field defect had almost resolved with only a left infero-temporal defect persisting. A pituitary MRI scan showed marked reduction in size of pituitary adenoma and drooping of the optic chiasm. Endocrine assessment revealed TSH, gonadotropin and partial ACTH deficiency. It was felt likely that he had developed pituitary infarction secondary to aortic dissection. He was established on appropriate hormonal replacement. He subsequently underwent aortic valve replacement and aortic repair under steroid cover. He remains stable although there has been intermittent fluctuation in visual symptoms.

Discussion

The partial hypopituitarism and shrinkage of the pituitary tumour in this case is likely to be explained by pituitary infarction caused by sudden alterations in critical perfusion pressure as a result of acute aortic dissection on a background of cardiac failure.

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P280

Isolated central hypoadrenalism as the sole manifestation of presumed neurosarcoidosis

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Introduction

Hypothalamic–pituitary sarcoidosis is uncommon and affects $<10\%$ of patients with neurosarcoidosis. It presents a diagnostic challenge. We present a case of isolated central hypoadrenalism presenting as the sole manifestation of neurosarcoidosis.

Case history

A 76-year-old man with recently diagnosed primary hypothyroidism presented with weight loss, increasing lethargy and fatigue. Physical exam revealed inguinal lymhadenopathy. Staging CT thorax showed bilateral mediastinal lymphadenopathy, interstitial lung parenchymal changes, and splenomegaly. Mild hypercalcaemia (calcium 2.65 mmol/l) and hyponatremia were noted.Initial differential diagnoses included lymphoma, malignancy, or granulomatous disease. Diagnostic excision biopsies of left inguinal and mediastinal lymph nodes were inconclusive. An empirical trial of steroid therapy for presumed sarcoidosis was commenced with symptomatic improvement in general well being. Withdrawal of steroids was attempted in view of the inclusive lymph node biopsies. This resulted in a marked deterioration in the patient with hypotension, listlessness and obtundation. Adrenal insufficiency was suspected. ACTH stimulation with tetracosactide (Synacthen) showed a sub-optimal cortisol increment with peak cortisol of 224 nmol/l. Adrenal antibodies were negative and serum ACTH level <5 mg/l suggesting secondary adrenal insufficiency This

was confirmed by a subsequent long ACTH stimulation test. CT imaging showed no gross pituitary abnormality. The patient improved symptomatically on glucocorticoid replacement therapy.
Discussion
The exact mechanism of injury in hypothalamic–pituitary sarcoidosis is unclear. Endocrine dysfunction was attributed to granulomatous infiltration of the hypothalamus and pituitary though hypothalamic insufficiency and pituitary responsiveness to hypothalamic releasing factors are now thought to be the main cause of hypopituitarism. Central hypoadrenalism can present with protracted and non-specific symptoms and pose a diagnostic challenge in the absence of any gross structural pituitary abnormality. A high index of suspicion remains the cornerstone of diagnosis.

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P281

Etiology and outcome of hyponatremia due to pituitary insufficiency in a tertiary endocrine center

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Background
Hyponatremia is a common electrolyte abnormality, especially in elderly, hospitalized patients, with a prevalence of severe hyponatremia (<125 mmol/l) up to 6–8%. Pituitary insufficiency (TSH+ACTH) may be difficult to diagnose, but it is important to differentiate it from SIADH and cerebral salt wasting syndrome.
Aim
To describe the aetiology and outcome of patients with hyponatremia due to pituitary insufficiency in a tertiary endocrine center.
Patients and methods
The records of 40 patients presented with/referred for hyponatremia (< 130 mmol/l) in the Department of Pituitary Pathology between 2005 and 2012, were retrospectively reviewed. There were identified 30 patients (16M/14F, aged 61.9±14.3 years) with hyponatremia due to pituitary insufficiency, three patients with severe primary hypothyroidism and seven patients with primary adrenal failure.
Results
In 13 patients, hyponatremia was the event revealing pituitary insufficiency; mean serum sodium at diagnosis was 113.7±8.6 mmol/l (range: 97–128). Severe hyponatremia (<125 mmol/l) was recorded in 26/30 patients (86.7%). Panhypopituitarism or multiple pituitary deficiencies were present in 28 out of 30 patients (93.3%); two patients (6.7%) showed isolated ACTH deficiency. Median 0800 h serum cortisol at the moment of diagnosis of secondary adrenal failure was 2.3 μg/dl (range: nd-12.34 μg/dl). The etiology of panhypopituitarism was: pituitary tumors ($n=18$), empty sella ($n=5$), Sheehan's syndrome ($n=4$ cases), possible autoimmune hypophysitis ($n=3$). There were 14 nonfunctioning pituitary adenomas, 1 acromegaly, 2 prolactinomas and 1 ACTH secreting adenoma. On first hospital admission, 5/30 patients were comatose and two patients had seizures. All patients recovered after saline infusion and steroid therapy; three patients were cautiously treated with infusions of 5.85% saline diluted in 0.9% saline. There were no fatalities or osmotic demyelization syndrome.
Conclusions
Hypopituitarism with TSH and ACTH insufficiency seems to be a frequent endocrine cause of severe hyponatremia. Correct diagnosis is important, as glucocorticoids are very effective.

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P282

Spontaneous resolution of pituitary Cushing's

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A 32-year-old male was referred by his GP with a 6-month history of lethargy, erectile dysfunction, weight gain, acne and hypertension. He denied exogenous steroid usage and had no other past medical history. On examination, he had classical features of Cushing's including moon face, central adiposity, proximal muscle weakness and purple striae.
Investigations confirmed Cushing's from a pituitary source as shown in the table below. MRI pituitary showed a probable pituitary microadenoma on the left side. His case was discussed at the pituitary MDT and was offered surgery. About 2 months before surgery, he developed sudden severe headache for which he attended A&E department where he was discharged after pain control. Following this episode his symptoms of Cushing's improved, started to lose weight. He underwent surgery but histology revealed normal pituitary tissue.
Three months after surgery, he had lost 10 kg in weight with a normalising body habitus. Blood pressure was also back to normal. A repeat low dose dexamethasone suppression test was normal with 0900 h serum cortisol of 12 nmol/l and his full pituitary profile was also normal. Repeat MRI pituitary showed a normal pituitary gland.
Conclusion
Spontaneous remission in pituitary Cushing's disease has been documented in very few cases. The possible etiology is considered to be possible infarction or haemorrhage into the adenoma. We suspect that he might have infarcted his pituitary shortly before his surgery when he attended the A&E with severe headache.

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P283

A case of complex neurodevelopmental abnormality causing asymptomatic SIADH

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Case
A 25-year-old lady was incidentally found to have hyponatremia while she was investigated for painful left ankle and hand swelling. At the time of review in endocrine clinic, she was completely asymptomatic. Her menstrual period was normal. **On examination** she was found to be hypertensive with consistent blood pressure of 188/110. Rest of the systemic examination was unremarkable. **Investigations** revealed biochemical findings consistent with SIADH. Her serum sodium was 121 mmol/l, potassium 4.4 mmol/l, urea 1.7 mmol/l and creatinine 72 μmol/l. Her serum osmolality was 254 mosmol/kg, urine osmolality 455 mmol/kg and urine sodium 44 mmol/l. Her serum cortisol was 862 nmol/l and TSH was normal. SIADH was confirmed by serum AVP level of 0.68 pmol/l which should be undetectable at serum osmolality of 254 mosmol/kg.
Her chest X-ray was unremarkable. MRI of the brain showed complex developmental anomalies with absence of corpus callosum, dilatation of occipital horn of right lateral ventricle and anteriorly situated inter hemispheric cysts which was thought to be the cause of SIADH.
She was treated with 1.5 l of fluid restriction to maintain her serum sodium between 125 and 131 mmol/l. Her blood pressure was treated with doxazosin 2 mg daily after ruling out phaeochromocytoma and primary hyperaldosteronism.
Conclusion
This rare case highlights the importance performing thorough neurological assessment of patients presenting with asymptomatic hyponatremia.

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P284

Hypopituitarism with visual field loss is not always an adenoma

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Introduction
A biochemical profile of an underactive pituitary gland allied to visual field loss is commonly due to an underlying adenoma. We report a case where such a clinical picture was found but imaging/biopsy revealed a different cause.
Case report
A 52-year-old Indian male was referred by his GP to an endocrine outpatient department with reduced visual acuity, balance problems and a biochemical profile of an underactive pituitary gland. Past history included hyperlipidaemia, allergic rhinitis and depression. Examination revealed a bitemporal hemianopia but no long tract neurological signs. Blood tests showed free T_4 of 6 pmol/l, TSH 0.26 ml/l, testosterone nul <0.1 ng/ml, prolactin 885 μ/l and IGF1 6.0 nmol/l. An MRI head showed a large ill-defined mass of the optic chiasm. This extended into the hypothalamus and was suspicious of an optic chiasm glioma. The patient was started on thyroxine, testosterone and hydrocortisone therapy. He was referred for neurosurgical opinion and a hypothalamus biopsy revealed the presence of granuloma. Cerebrospinal fluid culture is currently negative for TB but showed a raised protein level of 0.98 g/l. The patient was started on anti-TB medication (he had reported contact with a family member who had TB) and steroids (for the possible differential of sarcoid.) This patient continues to be cared for by the endocrinology, neurology, ophthalmology and respiratory teams.
Discussion
Visual field loss alongside a picture of an underactive pituitary gland is not always caused by an adenoma. In such cases where other causes are apparent, medical replacement therapy is merited alongside involvement of different specialists in the long-term management plan.

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P285

Rapidly progressive pituitary carcinoma in a young female

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A 32-year-old female presented with a 6-week history of worsening headaches associated with bitemporal visual field defects. An urgent MR scan demonstrated a sizeable pituitary lesion with chiasmal compression and suprasellar extension. Endocrine tests revealed hyperprolactinaemia (2550 mU/l) with hypocortisolaemia (<37 nmol/l). Cabergoline and hydrocortisone replacement were initiated. Unfortunately, 24 h later her visual fields deteriorated further and the patient underwent a subtotal transsphenoidal, followed by transcranial resection of the tumour. Post-operatively, the visual fields improved slightly. The histology was in keeping with carcinoma – either of metastatic origin or primary pituitary cancer. A CT chest, abdomen and pelvis did not suggest a possible primary.
Three weeks post-operatively, the patient re-presented with worsening headaches and a deterioration in visual fields. Tumour recurrence was confirmed on CT and the patient proceeded to have urgent radiotherapy. Unfortunately, 2 days after completing the course of radiotherapy (3 months post-surgery), she was readmitted to hospital with progressive headache, nausea and deterioration of vision in the left eye. An MRI demonstrated widespread meningeal thickening with evidence of multiple durally-based metastases. Upon review by the oncology team it was advised that the patient have a lumbar puncture for CSF cytology and a repeat CT chest, abdomen and pelvis to aid future management decision. Sadly however, this patient passed away days after this review.
This case not only highlights the difficulty in diagnosis but also the therapeutic challenge in managing what has been in this case a rapidly fatal disease.

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P286

Growth of a meningioma in a female patient with uncontrolled congenital adrenal hyperplasia

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Context
Growth of meningiomas has been previously described in patients receiving oestrogen/progestogen therapy.
Methods
Case history, laboratory findings, imaging and histology are discussed.
Case history
A 45-year-old woman with a known history of 21-hydroxyase deficiency (of the non-salt wasting variety) and long-standing non-adherence with corticosteroid therapy presented to the Endocrine Clinic for follow-up care. She complained of severe headache. On examination she was of short stature, virilised and had marked right sided proptosis.
Laboratory findings
Testosterone 19 nmol/l (0–1.5), 17-hydroxyprogesterone >180 nmol/l (<6.5).
Imaging revealed a large left sphenoid wing meningioma with anterior displacement of the right eye.
Histology
Meningioma, Ki index?, MIB-1 index? stained positive for progesterone receptors
Discussion
Growth of meningiomas has been described in patients receiving menopausal hormone therapy, long active reversible contraceptives[1] and transsexual patients[2]. To our knowledge this is the first reported case of aggressive meningioma putatively related to stimulation of the progesterone receptor in response to high levels of 17-hydroxyprogesterone in a patient with uncontrolled congenital adrenal hyperplasia.

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P287

Finally we know! 'It comes from your pituitary'

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Introduction
Cushing's syndrome is broadly categorized into ACTH dependent (pituitary and ectopic source) and ACTH independent (adrenal source). Localizing source of Cushing's can sometimes be a cumbersome diagnostic process.
Case history
A 25-year-old male patient presented with sudden rapid onset weight gain, muscle weakness and occasional headaches.as well as severe dyspnoea, orthopnea and PND. There was no significant past medical or family history. He was not on any regular medication and denied exogenous steroids intake. He had clinical features consistent with florid Cushing's syndrome and congestive cardiac failure. Cardiac MRI suggested severe dilated Cardiomyopathy with EF 23% which was treated medically. His screening investigations for Cushing's showed discordance with clinical picture. He had high 02400 h urine free cortisol on two occasions but suppressed <28 on low dose dexamethasone suppression test. Two early mornings ACTH levels were undetectable and prompted investigations to find an adrenal source. CT as well as MRI adrenals failed to localize an adenoma. Alternative sources were then explored. Pituitary MRI and subsequent dynamic pituitary MRI were entirely normal apart from stalk deviation to left side. No ectopic source of disease was found on Gallium octreotide PET–CT. Rest of the Pituitary function tests were satisfactory. He had Inferior petrosal sinus sampling which showed strong lateralization to left side of pituitary. He is currently awaiting pituitary surgery for Cushing's disease and has been started on blockade therapy with Metyrapone in the interim. Repeat cardiac MRI shows improvement in cardiac function (EF 41%).
Conclusions
Diagnosing Cushing's syndrome and identifying the source can sometimes be challenging and require more invasive investigations. We highlight importance of taking clinical picture into account whenever dealing with complicated Cushing's patients and their discrepant investigations.

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Reproduction

P288

Inflammatory markers in polycystic ovarian syndrome and their association with cardiovascular risk factors

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Objectives
To determine and compare inflammatory markers including adiponectin, visfatin and IL18 in patients with polycystic ovarian syndrome (PCOS) 2. To find out whether adiponectin and interleukin 18 (IL18) is associated with markers of insulin resistance, hyperandrogenism and carotid intima-media wall thickness (CIMT) as a cardiovascular risk factor.
Methods
This is a prospective controlled study involving 60 consecutive euglycemic patients with PCOS (Rotterdam criteria) and 50 age and BMI matched controls were included in the study. After detailed clinical evaluation including anthropometry, besides oral glucose tolerance test, fasting venous samples were analysed for IL18, visfatin, adiponectin, highly sensitive C-reactive protein (hs CRP) and complete lipid profile. We estimated body composition (total body fat and visceral adipocity index, VAI by dual energy X-ray absorptiometry), CIMT (by Doppler ultrasonography), indices of insulin sensitivity (QUICKI) and resistance (homeostasis model assessment for insulin resistance, HOMA-IR) and free androgen index (FAI). Data were analyzed using online graphpad quickcalc software and $P<0.05$ was considered statistically significant.
Results
PCOS patients had greater FAI (1.42 ± 0.83 vs 0.64 ± 0.4), higher HOMA-IR (2.13 ± 1.05 vs 1.91 ± 1.8) and lesser QUICKI (0.156 ± 0.025 vs 0.163 ± 0.015) than the control groups. Patients with PCOS have significantly increased serum IL18 and visfatin levels than that of the control group (IL18: 213.48 ± 76.84 vs 170.4 ± 41.11 pg/ml, visfatin: 73.35 ± 11.54 vs 55.56 ± 9.27 ng/ml, $P<0.05$) and hsCRP (2.56 ± 0.64 vs 1.62 ± 0.78 mg/l, $P=0.004$). Similarly the PCOS group had significantly lower level of adiponectin (0.8 ± 0.6 vs 1.04 ± 0.49 ng/ml, $P<0.001$). Correlation coefficients of IL18 were as follows: with CIMT (0.355), FAI (0.328), HOMA-IR (0.345) and waist circumference (0.367), each with $P<0.05$. Similarly the correlation coefficients of adiponectin were with CIMT (-0.312), FAI (-0.343), HOMA-IR (-0.352) and waist circumference (-0.359), each with $P<0.05$.
Discussion
There is alteration of adipokines and other inflammatory markers in PCOS with increase in visfatin, IL18 and hs CRP and reduction in of adiponectin levels. Increased IL18 and decreased adiponectin levels correlated with insulin resistance, obesity and hyperandrogenism.
Conclusion
These altered adipokine profile is associated with increased CVD risk in PCOS patients, leading to the suggestion that one of these markers like IL18 can serve as potential therapeutic target in future for decreasing their CV risk.

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P289

Maternal corticosterone regulates amino acid allocation to fetal growth in mice

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Synthetic glucocorticoid administration during pregnancy reduces birth weight and increases disease risk in the offspring. This study investigated whether increasing maternal natural corticosterone levels, by endogenous stress or exogenous administration, alters fetal amino acid supply in the mouse.
C57BL6/J dams ($n=82$) were exposed to daily restraint and subcutaneous saline injection (stress) or given corticosterone (cort, 82 ± 11 μg/g per day in drinking water) for 5 days in mid/late pregnancy (either D11–16 or D14–19, term is D21). Plasma corticosterone was measured by RIA. On D19, placental amino acid transport was determined as the materno-fetal clearance of non-metabolisable ^{14}C-methylaminoisobutyric acid (MeAIB). Gene expression was determined in snap-frozen placentae using qPCR. Significant effect ($P<0.05$) of treatment was determined by one way ANOVA with Bonferroni's *post-hoc* test.
Compared with untreated controls (ut), stress or cort from D11–16 increased plasma corticosterone on D16 (ut, 571 ± 58 ng/ml; stress, 1018 ± 109 ng/ml; cort, 1142 ± 134 ng/ml; $P<0.05$) but not on D19 (ut, 714 ± 41 ng/ml; stress, 739 ± 53 ng ml; cort, 524 ± 53 ng/ml; $P>0.05$). Corticosterone remained was high on D19 in both groups of dams treated from D14–19 (stress, 949 ± 134 ng/ml; cort, 1143 ± 176 ng/ml. $P=0.003$). On D19, MeAIB clearance was affected by treatment ($P=0.007$), and was highest in D11–14 cort dams and lowest in D14–19 cort dams. When all groups were combined, MeAIB clearance inversely correlated with maternal corticosterone (Pearson's $r=-0.50$, $P<0.001$). On D19, placental *Redd1/Ddit4*, an inhibitor of mammalian target of rapamycin (mTOR) signalling, was upregulated when maternal corticosterone was high in D14–19 cort treated dams (142% vs ut, $P<0.05$) but did not differ from control values in D11–16 cort dams.
The results show that physiological increases in corticosterone during mouse pregnancy regulate fetal amino acid supply, in part, through inhibition of placental mTOR signalling. This effect may defend amino acids for maternal metabolism, at the expense of fetal growth.
Declaration of funding
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P290

BMP4 induces terminal differentiation of primary trophoblast cells and increases chorionic gonadotrophin secretion

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Objective
Chorionic gonadotrophin (CG) is a hormone induced during terminal differentiation of trophoblast cells that significantly influences pregnancy outcome. The TGFβ superfamily SMAD2/3 pathway regulates placental function but the activity of the alternative pathway through SMAD1/5/8 in the placenta is unknown. This study investigated the role of BMP4 signalling through SMAD1/5 in terminal differentiation of primary chorionic gonadotrophin-secreting trophoblast cells.
Methods
A novel equine animal model of trophoblast differentiation was used to gain pure populations of primary CG-secreting trophoblast cells and placental tissue. Chorionic girdle was isolated from days 27 to 34 equine conceptuses. A 44 K gene probe equine expression array and RT-PCR was used to compare Type I and Type II serine/threonine kinase and accessory receptor expression. Cultured chorionic girdle trophoblast cells were supplemented with 1–100 ng/ml human BMP4. Differentiation was determined following dual-labelling of the cells with CellTrace BODIPY TR methyl ester and Hoeschst. eCG concentration was determined using ELISA. Total SMAD1/5, pSMAD1/5, total SMAD2 and pSMAD2 expression in the placenta was determined using western blotting.
Results
Chorionic girdle tissue and cultured CG-secreting trophoblast cells preferentially expressed receptors ALK3, BMPR-II, Dragon and Bambi that bind the ligand BMP4. Stimulation of chorionic girdle trophoblast cells with 1–100 ng/ml BMP4 resulted in a dose dependent increase in total number and proportion of terminally differentiated binucleate cells ($P<0.001$) and induced eCG secretion ($P<0.01$) in a developmental dependent manner. Phospho-SMAD1/5 expression, but not pSMAD2 was tightly regulated during CG-secreting trophoblast differentiation *in vivo*, with peak expression of pSMAD1/5 noted at gestation day 31 corresponding to maximal trophoblast differentiation.
Conclusion
Our findings support a role for TGFβ signalling in regulation of differentiation of primary trophoblast cells via BMP4 dependent binding to BMPR-II and ALK3 and activation of SMAD1/5. The observation of BMP4 signalling in primary trophoblast provides a previously unreported mechanism of TGFβ signalling in the placenta.
Declaration of funding
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P291

Is diethylstilboestrol an endocrine disruptor in the developing human fetal testis? Effects of DES exposure using a xenograft approach

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Context
In rodents, *in-utero* exposure to the exogenous oestrogen diethylstilboestrol (DES) results in reproductive abnormalities in male offspring. It has been

proposed that similar anti-androgenic effects also occur in the human fetal testis following oestrogen exposure.
Objective
Determine effects of DES exposure on testosterone production by normally growing human fetal testis xenografts.
Design
Human fetal testes (15–19 weeks gestation, $n=6$) were xenografted into castrate male nude mice. Mice were treated for 35 days with vehicle or 100 μg/kg DES three times a week; all mice were treated with hCG to mimic normal human pregnancy. For comparison, fetal male rats were exposed *in-utero* to vehicle or 100 μg/kg DES on e13.5, e15.5, e17.5, e19.5 and e20.5.
Main outcome measures
Testosterone production from xenografts was assessed by measuring host serum testosterone and seminal vesicle (SV) weights at termination. Insl3 production was determined in serum, whilst immunohistochemistry was performed for Insl3 and ERα.
Results
Human fetal testis xenografts showed similar survival and total graft weight in DES and vehicle-exposed hosts. SV weight was significantly increased in DES compared to vehicle-exposed hosts (44.3 vs 26.6 mg, $P=0.01$) and testosterone was also increased (0.33 vs 0.45 ng/ml, $P>0.05$). There was no difference in Insl3 expression. In contrast, *in-utero* exposure of fetal rats to DES resulted in significantly lower intra-testicular testosterone levels associated with a reduction in Insl3 expression. ERα was expressed in fetal rat testis but was not expressed in the human fetal testis.
Conclusions
Exposure of human fetal testes to DES does not impair testosterone production as it does in rats. This can be explained by a lack of ERα signalling in the human fetal testis. This highlights an important difference in the effect of proposed 'endocrine disruptors' in humans compared with rodents which has important implications for determining the risk posed to human health by environmental oestrogens.
Declaration of funding
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P292

Perinatal origins of adult Leydig cells and function: role of developmental androgens

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Fetal events can affect adult testosterone levels but how this occurs is unknown, as adult Leydig cells (ALC) do not differentiate until puberty. Qin *et al*. 2008 (*PLos ONE*) identified that chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) is essential postnatally for ALC development. We hypothesized that: i) COUP-TFII$^+$ non-Leydig interstitial cells are progenitors for ALC and are present in the fetal testis, ii) these 'progenitor ALC' are regulated by fetal androgens, and this might affect testosterone levels in adulthood.
Three approaches were used: i) complete androgen receptor (AR) knockout (ARKO) mice, ii) pregnant rats treated with dibutyl phthalate (DBP; 500 mg/kg per day; e13.5–e21.5) which reduces fetal intratesticular testosterone $>70\%$, iii) iNOS$^{-/-}$ mice in which intratesticular testosterone is increased. Numbers of progenitor ALC were quantified from fetal life to adulthood, and related to ALC number and function, (testosterone and LH levels, ALC-specific steroidogenic gene expression).
Presumptive progenitor ALC expressing AR (COUP-TFII$^+$/AR$^+$) are abundant in the fetal testis of rats, mice, marmosets and humans. In ARKOs, progenitor ALC number was reduced $\sim$40% from birth to adulthood, paralleled by a similar shortfall in ALC; there was also compensated ALC failure. In adults progenitor and ALC numbers were correlated ($P<0.0001$). Fetal DBP exposure reduced progenitor ALC numbers by $\sim$40% in fetal and postnatal life and induced compensated ALC failure and reduced steroidogenic gene expression. The iNOS$^{-/-}$ mice are currently under investigation.
This study suggests that COUP-TFII identifies a population of cells in the fetal testis from which ALC develop from puberty onwards, and these cells are partly regulated by androgens. Reduced fetal androgen exposure altered ALC function (mechanism unclear), presumably by altering fetal androgen action on the ALC progenitors. This adds a new dimension to growing evidence that fetal androgen exposure may determine functional competence of the adult testis and overall male reproductive health.
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P293

Pulsatile GnRH signaling to ERK: relevance of pulse duration and frequency

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GnRH is secreted in pulses and its effects on pituitary gonadotropes depend on pulse frequency. This is crucial for physiological control and therapeutic manipulation of the system (in IVF and treatment of hormone-dependent cancers) but GnRH pulse frequency decoding mechanisms are unknown. The simplest form of frequency dependence is a linear relationship between integrated inputs and outputs but such 'integrative tracking' cannot explain the bell-shaped frequency-response relationships seen for many GnRH effects. GnRH acts via Gq/11 coupled GPCRs to activate effectors including ERKs, which mediate many transcriptional effects of GnRH but little is known about ERK signaling with pulsatile stimulation so we have explored this with automated fluorescence microscopy in HeLa cells transduced with adenovirus expressing ERK2-GFP (1). Five minute GnRH pulses caused rapid, transient and reproducible ERK2-GFP activation (nuclear translocation) at varied pulse concentrations (0.01–100 nM) and frequencies (0.25–2/h). Using an Egr1 luciferase reporter, increasing pulse frequency increased the transcriptional response (to ERK activation) but increasing pulse duration had a less pronounced effect (i.e. Egr-1 luciferase was approximately doubled by doubling frequency from 0.5 to 1 pulse/h, but a similar effect required a 10$\times$ increase in pulse duration from 1 to 10 min). Exploring activation of endogenous ERKs revealed that ppERK1/2 levels continue to rise for at least 3 min after a 1 min GnRH pulse, which may explain the unexpectedly high transcriptional response to very brief stimulation. Thus, varying pulse frequency implies that the ERK pathway is a simple integrative tracker of GnRH pulse frequency, but varying pulse duration reveals it is not. ERK activation is more sensitive to pulse frequency than it is to pulse duration (in this short time-frame) and this may have adaptive advantages for use of the ERK pathway in frequency-encoded signaling system.
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P294

Intrahepatic cholestasis of pregnancy levels of sulfated progesterone metabolites downregulate hepatic LXRα

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Introduction
Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific liver disorder which is associated with higher incidence of gallstone disease. ICP symptoms are usually presented in the third trimester of gestation and their severity advances in parallel with the increase in serum sulphated progesterone metabolites (P4-S) in the mother. Liver X receptor α (LXRα) actively participates in the regulation of lipid metabolism functioning as a cholesterol sensor. We aimed to investigate if ICP levels of P4-S could modulate the LXRα transcriptome and thus contribute to gallstone formation by increasing biliary cholesterol sectretion.
Methods
The influence of ICP levels of P4-S on LXRα as well as its target genes was assessed in human hepatoma cells using RT-PCR and western blotting. LXRα reporter assays were employed to determine which domain of the nuclear receptor mediates the impact of the sulphated progesterone metabolites.
Results
Lucifease reporter assays demonstrated that the P4-S epiallopregnanolonesulphate (PM5S), epiallo-pregnanediol 3-sodium sulphate (EPAS) and epipregnanolonesulphate (EPS) were able to attenuate the basal as well as the agonist-induced transactivity of LXRα in a dose-dependent manner. Also, PM5S, EPAS and EPS were able to specifically modulate the activity of the LXRα ligand binding domain$\pm$the hinge region as shown using recombinant GAL4-LXRa vectors. Quantitative RT-PCT showed that PM5S, EPAS and EPS could decrease

the mRNA levels of the LXRα target ABCG1in a dose-dependent manner while the gene expression of of LXRα itself is reduced by PM5S only. Protein analysis also showed that PM5S, EPAS and EPS diminish the levels of LXRα in the nucleus.
Conclusion
Murine studies have shown that the lack of ABCG1 causes increased biliary cholesterol secretion which is a key pathophysiological event in the development of gallstones. Supraphysiological levels of P4-S contribute to the formation of gallstones in ICP by dowregulating LXRα and its target ABCG1.
Declaration of funding
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P295

DNA methyltransferase 3a, 3b and 3L expression in fetal germ cells and its modulation

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Background
5-Cytosine methylation of DNA is a means of encoding epigenetic information. In the testis, the generation of *de novo* methylation is conducted by the enzymes DNMT3a and 3b and the co-enzyme DNMT3L. Epigenetic marks made to the DNA of germ cells are important as a potential means of trans-generational carriage of environmental information. In fetal life, germ cell demethylation and remethylation are important physiological events and these overlap with key changes in germ cell differentiation (loss of pluripotency), but whether this is coincidental or not is unknown. This study characterised expression of the DNMT3s.
Methods
DNMT 3a, 3b and 3L were co-localised with the germ cell markers Oct4 and VASA using immunofluorescence in fetal testes from the rat (17.5 and 21.5 days post conception (dpc)), marmoset (98 and 110 dpc) and human (gestation weeks 14 and 19) to determine changes in expression related to age and to germ cell differentiation status.
Results
DNMT3a and 3b are expressed in some but not all germ cells of the fetal testis across all the species examined. DNMT3a is expressed in fewer pluripotent Oct4+ cells than DNMT3b. The proportion of Oct4+ germ cells expressing DNMT3b increased in the rat, marmoset and human from dpc 17.5 to 21.5, 98 to 110 and gestational weeks 14 to 19 respectively. Ongoing studies are characterising DNMT3L expression and identifying if there is a relationship between DNMT3 expression and germ cell differentiation.
Discussion
We show that DNA methytransferase enzymes are present in fetal germ cells across multiple species, including primates, at the protein level. The potential manipulation of DNA methylation by environmental stimuli is a mechanism by which life style and pathogen exposure could impact upon the health of subsequent generations. The presence of DNMTs in fetal germ cells demonstrates a means by which *de novo* cytosine methylation can be induced.
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Dissecting the prokineticin receptor dimerization interface: a role in kallmann sindrome?

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Prokineticin receptors (PKR1 and PKR2) are GPCRs that belong to neuropeptide Y receptor class. They exert their biological functions binding two structurally related peptides (Bv8 or PK2 and EG-VEGF orr PK1). Intensive research over the past few years has shown that PKs/PKRs signalling modulates neuronal survival and neurogenesis, hypothalamic hormone secretion, nociception, circadian rhythm and complex behaviours, such as feeding and drinking. It also promotes angiogenesis in steroidogenic tissues and reproductive organs, hematopoiesis and immune response.
A growing body of evidence points to the fact that GPCRs exist as homo- or heterodimers but the functional impact for many of these dimers still remains unclear.
The study's aim was to confirm PKR2 homodimerization, to indentify the homodimerization interface and to assess PKR1-PKR2 heterodimerization. Techniques namely bioluminescence resonance energy transfer (BRET) were used to study receptor-receptor interactions in live cells in real time. The interaction site of PKR2 homodimer was assessed by use of receptor fragments corresponding to TM1-5, TM1-4, TM1-7 and TM6-7. These were also employed to determine the role of PKR2 homodimerization in receptor targeting to the membrane.
Two missense mutations (P290S and L173R) within transmembrane domains of PKR2 sequence were also studied. It has been demonstrated that these PKR2 mutants can cause the Kallmann syndrome phenotype, which combines hypogonadism, due to gonadotropin-releasing hormone deficiency, and anosmia or hyposmia, due to defective olfactory bulb morphogenesis. We investigated the effects of these mutations on cell surface targeting and dimerization of both mutated and wild-type receptors.
Given the importance of dimerization in receptor synthesis and cell surface targeting, the work obtained can provide a framework for interpretation of results concerning the PKR2 mutants associated with Kallmann syndrome.
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P297

Tocopherol interacts with β-mimetic effect in reproductive and respiratory tracts in the rat

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Background
It is known that reactive oxygen species (ROS) production is necessary for the signal transduction mechanism of β-adrenergic receptors.
Aim
Our aim was to investigate whether antioxidant agent (α)-tocopherol-acid-succinate may reduce the effect of β-mimetic action on β(2)-adrenergic receptor regulated tissues.
Methods
Tissue samples were collected from non-pregnant (trachea) and 22-day pregnant (myometrium, cervix) rats. In separated groups of animals, the tracheal and uterine β-adrenoceptor activities were upregulated by 17-β-oestradiol-valerate and progesterone treatment, respectively. Cervical resistance, myometrial and tracheal contractions were investigated in isolated organ bath. The cyclic AMP accumulations in the tissues were measured with competitive cyclic AMP Enzyme Immunoassay (EIA) Kit.
Results
Terbutaline (10^{-6} M) increased the cervical resistance both in non-treated and progesterone-treated samples. Tocopherol (10^{-7} M) reduced this action in progesterone-treated samples, but had no effect on non-treated cervices. Terbutaline (10^{-10} to 10^{-5} M) decreased the spontaneous myometrial contraction both in non-treated and progesterone-treated tissues. The presence of tocopherol did not alter this action in any cases. Terbutaline (10^{-9} to 10^{-4} M) reduced the tracheal tones both in non-treated and oestrogen-treated tissues. This effect was reduced by tocopherol in both cases (by 52% in non-treated and 68% in oestrogen-treated tissues). The changes in tissues cAMP levels showed a strong correlation with the isolated organ results.
Conclusions
Tocopherol as a free radical scavenger may interact with the β-mimetic action. This effect is pronounced in trachea, slight in the cervix and non-significant in the myometrium. These results suggest that the ROS may have different significance in the signalling process of β(2)-adrenergic receptors in different tissues.
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P298

Steroid regulation of gene and protein expression of osteopontin and αvβ3 integrin in ovine endometrium

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At implantation, the ability of an embryo to successfully attach to the luminal epithelium is dependent on the receptive phenotype the endometrium must acquire. This spatially and temporally restricted period of uterine receptivity is defined by endometrial molecular adaptations, highly regulated by embryonic-derived signals and ovarian steroids. Critical to this isthe expression of adhesivemoleculesintegrins and osteopontin (OPN) at the foetal-maternal interface which facilitate initial embryo-endometrial interactions. Osteopontinis the primary ligand for αvβ3 integrinwhich show coordinateincreased mid-luteal expression. Aims of this study were to characterise spatio-temporal expression and steroidal regulation of osteopontin and αvβ3 in ovine endometrium.
Sheep uteri obtained from intact mid-luteal and follicular phase and from ovariectomised ewes subjected to intramuscularinjections of 12.5 mg/ml progesterone ± 3 μg/ml oestradiol for 10 days, commencing 6 days following ovariectomy. Molecular analysis of osteopontin and αvβ3 and its αv and β3 subunits was performed by RT-PCR and immunohistochemistry.
Cycle-dependent changes in osteopontin and αvβ3 were confirmed, with an increase in OPN and β3 subunit in mid-luteal phase endometrium.Peak levels of OPN transcript notable in mid-luteal inter-caruncularissue, indicates abundance of glands in these regions are likely the primary source of OPN endometrial production. Progesterone treatment induced an increase in OPN mRNA and protein, most significantly in glandular epithelium. The cellular distribution of αv subunit was comparable to osteopontin, although levels of transcript and protein were shown to peak in follicular phase and were induced most significantly in response to oestradiol. The spatial and temporal distribution pattern of β3 subunit differed to αv with no detectable staining in either luminal or glandular epithelium, but strong immunoreactivity was consistent in both phases in vasculature and increasedin subepithelialstroma in mid-luteal phase compared to follicular. In conclusion, mid-luteal up-regulation of OPN and αvβ3 may facilitate early embryo attachment.
Declaration of funding
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P299

The effect of mTOR blockers on japanese quail ovarian granulosa cell functions

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The aim of our study was to elucidated the role of mTOR-dependent intracellular signalling pathway in control of ovarian functions. For this purpose, we have examine the effect of three mTOR inhibitors (resveratrol, curcumin and synthetic mTOR blocker MC 2183 – Mai *et al*. 2005[1], at the doses 0, 1, 10, 100 μg/ml) on apoptosis and steroidogenesis by cultured Japanese quail ovarian granulosa cells. The release of steroid hormones (progesterone and testosterone) and accumulation of bax (marker of apoptosis) was analysed by RIA and immunocytocxhemistry respectively.
It was observed, that resveratrol adition decreased progesterone release (at 1 and 10 μg/ml but not at 100 μg/ml) and stimulated testosterone release (at 10 and 100 μg/ml but not at 1 μg/ml), as well as increased the percentage of apoptotic (bax-positive) cells at dose-dependent manner at all doses (1, 10 and 100 μg/ml) added. Curcumin treatment diminished progesterone release (at 10 and 100 μg/ml but not at 1 μg/ml), activated both testosterone release (at 10 and 100 μg/ml but not at 1 μg/ml) and apoptosis at all doses (at 1, 10 and 100 μg/ml). MC 2183 addition significantly down-regulated progesterone secretion (at 1 and 100 μg/ml but not at 10 μg/ml), did not affected testosterone release at any dose (at 1, 10 and 100 μg/ml) and increased accumulation of bax (at 10 and 100 μg/ml but not at 1 μg/ml).
These observations suggest the involvement of mTOR-dependent intracellular pathway in control of ovarian steroidogenesis and apoptosis. It can be involved in promotion of progestogen, inhibition of androgen and suppression of apoptosis in avian ovarian cells.
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P300

K_{ATP} channels are involved in the tocolytic effect of β_2 agonists in pregnant rat

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Preterm birth defined is a major determinant of neonatal mortality and morbidity. The incidence of preterm birth has not decreased over the years despite major improvements in medical research. In the view to decreasing the potentially maternal and foetal adverse events it is a pharmacological challenge to find new therapeutic strategies. In the clinical practice the most frequently used tocolytic agents are the β_2-adrenoceptor agonist (terbutaline, fenoterol, ritodrine).
Present study unravels the functional presence of ATP-sensitive potassium channel (K_{ATP} channel) and its involvement in mediating β_2-adrenoceptors-induced myometrial relaxation in rat myometrium at 6 and 22 days of gestation. The tissues pretreated with 10^{-6} M glibenclamide (K_{ATP} channel blocker), the relaxant effect of β_2 agonists were significantly lower compared with alone and the dose-response curves were shifted to right at day 6 of pregnancy, while the glibenclamide was ineffective at day 22. The combination of β_2 agonists with pinacidil (K_{ATP} channel opener) the uterus-relaxant effect dose-dependently increased at day 6 of pregnancy. At term the synergism between β_2 agonists and pinacidil was not observed. The ontogeny of K_{ATP} channel subunit (SUR1) was investigated by RT-PCR and western blot techniques; high level was found at 6 day of pregnancy and low level was detected at term. From these results it can be concluded: there is a close correlation between K_{ATP} channel density and β_2-adrenoceptor induced K_{ATP} channel activation in the rat myometrium.
Results of our study evidently suggest the functional presence of K_{ATP} channel in the pregnant rat myometrium and the role of β_2-adrenoceptor agonist induced myometrial relaxation at early stage of pregnancy. Based on these findings, it may not be suggest that the therapeutic application of both the β_2-adrenoceptor agonist and specific K_{ATP} channel opener as promising tocolytic agent. However, it may be used for treatment of habitual abortion.
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P301

Possible role of fetuin-B in the preterm delivery in the rat

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Fetuin-B is an inhibitor of basic calcium phosphate, preventing unwanted calcification. The low level of fetuin could be associated with an increased risk of atherosclerosis and ectopic microcalcifications in soft tissues and the rupture of the membranes leading to preterm delivery. Our aims were i) to examine the alterations of mRNA expression of the fetuin-B in the late pregnant rat uterus, furthermore ii) to investigate the fetuin-B expression in hormonally- and lipopolysaccharide (LPS)-induced preterm labour. iii) Moreover we examined the effect of farnesoid X receptor (FXR) agonist ursodeoxycholic acid (UDCA) on fetuin-B expression.
Changes in fetuin-B mRNA expression were measured by real-time PCR on pregnancy days 18, 20, 21 and 22 in rat uterus and after induction of preterm delivery. From pregnancy day 18 the animals were treated with UDCA (100 mg/kg per day).
The fetuin-B mRNA expression was the highest on days 20 and significantly decreased by last day of pregnancy (day 22). In hormonally-induced preterm labour, the mRNA expression of fetuin-B was markedly reduced similarly to the last day of normal pregnancy. On the other hand, the fetuin-B expression increased in the inflammatory preterm birth induced by LPS.
The pre-treatment with UDCA significantly increased the fetuin-B mRNA level on day 22 and in LPS-induced preterm birth. In contrast, UDCA did not alter the mRNA level in hormonally-induced preterm model.
We suppose that the altered expression of fetuin-B may have importance in the initiation of delivery in rat and may serve as a marker to indicate the possible reason of the preterm birth. Further studies are required to clarify the putative role of FXR agonists in the control of delivery or preterm birth.
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P302

Effect of t-2 toxin and combination of t-2 toxin with resveratrol and mc2183 on ovarian cells of japanese quails

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Occurence of mycotoxins in various food and feed commodities is a worldwide problem. T-2 toxin is one of the most toxic trichothecene mycotoxin, produced mainly by *Fusarium* species. Poultry belongs to very sensitive species which are very often explored to toxic effects of mycotoxins. The aim of our *in vitro* studies was to examine secretion activity of ovarian granulossa cells to produce progesterone P_4 after T-2 toxin and its combinations with resveratrol (RSV) and synthetic mTOR blocker MC2183 addition. Ovarian granulosa cells were incubated without (control group)/with treatments of natural substances at various doses for 24 h: T-2 toxin (10 100 1000 ng/ml), T-2 toxin (10 100 1000 ng/ml) plus resveratrol (60 μg/ml) and T-2 toxin (10 100 1000 ng/ml) plus MC2183 (60 μg/ml). Secretion of progesterone after addition of T-2 toxin and combinations of T-2 toxin with resveratrol or MC2183 was determined by RIA. Progesterone release by GC was stimulated by T-2 toxin at the doses 1000 ng/ml but P_4 was inhibited by addition at 100, 10 ng/ml. Stimulation of P_4 output at all used doses was after addition of combination of T-2 toxin (10 100 1000 ng/ml) plus RSV (60 μg/ml). On the other hand, inhibitory effect was after addition of T-2 toxin (10 100 ng/ml) in combination with MC2183 (60 μg/ml). However at highest used doses T-2 toxin (1000 ng/ml) plus MC2183 (60 μg/ml) we observed stimulation in P4 release by ovarian granulosa cells. All our observed results were not significant ($P \geqq 0.05$). In conclusion, our results suggest possible effect of T-2 toxin and its combination with RSV or MC2183 on ovarian function.

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P303

***In vitro* effect of 4-nonylphenol and 17β-estradiol on bovine spermatozoa**

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Nonylphenol (NP) is an endocrine disruptor that is capable of interfering with the hormonal system of numerous organisms. Estrogens play a central role in female reproduction, but also affect the male reproductive system. In males, they stimulate sperm capacitation, acrosome reaction and fertilizing ability. The aim of the present study was to investigate the effect of 4-nonylphenol (4-NP) and the effect of 17β-estradiol (E_2) on bovine spermatozoa motility. We examined the dose- and time-dependent effect of 4-NP (1, 10, 100 and 200 μg/ml) with and without addition 1 μg/ml E_2 on the spermatozoa motility during several time periods (0, 2, 4 and 6 h). The spermatozoa motility was determined by Computer Assisted Semen Analyzer (CASA) system using the Sperm Vision program and the percentage of motile spermatozoa (motility >5 μm/s) was evaluated. This study was performed in 10 replicates at each concentration. At least 1000 spermatozoa were analyzed in each sample. The control group (medium without NP) was compared to the experimental groups (exposed to different concentrations of NP), to the positive control group (medium with E_2) and to the experimental groups (exposed to different concentrations of NP with addition E_2). The obtained data showed a decreased motility of bovine spermatozoa in all experimental groups with the addition of 4-NP. NP significantly ($P<0.001$ and $P<0.05$) decreased spermatozoa motility in all experimental groups. The lowest spermatozoa motility ($P<0.001$) was found at doses >100 μg/ml of NP in comparison with the control group during all time periods. The addition of 17β-estradiol significantly ($P<0.05$) increased the motility in the experimental groups with 10, 100 and 200 μg/ml of NP during 4 and 6 h of cultivation. In conclusion, our results confirm that the high doses of 4-NP have the negative effect on spermatozoa motility, but E_2 can have the stimulating effect on spermatozoa motility.

4-nonylphenol, 17β-estradiol, bovine spermatozoa, motility, CASA system.

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P304

Effects of iron on the steroidogenesis of human adrenocarcinoma (nci-h295r) cell line *in vitro*

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Currently, there is increasing evidence that various chemicals introduced in the environment have the potential to cause damage to endocrine system, which regulates reproductive processes. Iron has various effects on reproductive endocrinology and it can also cause or contribute to hormonal disruption and to interfere with the key enzymes involved in steroid synthesis. The target of this *in vitro* study was to determine the effects of iron ($FeSO_4 \cdot 7H_2O$) on the steroidogenesis in the human adrenocortical carcinoma (NCl-H295R) cell line, which serves as a model system for screening endocrine-disruptive chemicals. The NCl-H295R cell line was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were cultured in the presence of iron (3.9; 62.5; 250; 500; 1000 μM $FeSO_4 \cdot 7H_2O$) or without $FeSO_4 \cdot 7H_2O$ (the control group) for 48 h. ELISA was used for the steroid hormones – testosterone (T) and progesterone (P) quantification directly from the culture medium. A concentration-dependent depression in the testosterone production was observed at the highest concentrations (≥ 250 μM) of $FeSO_4 \cdot 7H_2O$. The groups with the lowest doses (3.9–62.5 μM) stimulated the release of testosterone by the NCl-H295R cell line. The progesterone production was also decreased at the highest concentrations, but this decline was less evident in comparison to the testosterone decrease. The highest concentration of progesterone was significantly ($P<0.001$) detected at lowest dose (3.9 μM) of $FeSO_4 \cdot 7H_2O$. Results of this study showed a dose-dependent decrease of the steroid producing cells at very high concentrations of iron and subsequent changes in the concentration of testosterone and progesterone by adrenocortical carcinoma cells. Iron at low concentrations stimulated the steroid hormones synthesis, which presumably can affect also their metabolites or enzymatic pathways.

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P305

Gaining a better understanding of individual experiences of weight regulation in polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is an endocrinopathy affecting 5–10% of women. The PCOS symptomatology spectrum includes compromised fertility, dermatological conditions and metabolic dysregulation, the predominant cause of which is excess androgen production. PCOS is associated with increased risk of developing features of the metabolic syndrome which is exacerbated by the fact between 40 and 80% of PCOS diagnosed women are estimated to be obese. Achieving modest weight loss can improve fertility and dermatological conditions including acne and hirsutism, however for many PCOS diagnosed women losing weight and/or sustaining weight loss is challenging. Individual experience of PCOS symptomatology and its milieu, which has consequences for quality of life and emotional wellbeing, may itself be a promoter of weight gain and a barrier to weight loss. In a recent study examining female reproductive and metabolic health, women of reproductive age were invited to answer questions about their general, reproductive and metabolic health. Following the initial data collection phase, follow up interviews were carried out with women diagnosed with PCOS to examine in depth their experiences managing their weight and investigate common predictors of weight gain. Themes reported include control over body weight, lifestyle practices adopted to maintain weight, knowledge of energy balance regulation, sources of support and impact on emotional wellbeing. This on-going work will contribute to the knowledge and understanding of the health service needs of obese women diagnosed with PCOS and identify if specific factors should be considered when designing future strategies for intervention.

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P306

Regulation of LH/CG receptor signaling in human endometrium and perturbations in recurrent pregnancy loss

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G-protein coupled receptors (GPCRs) represent the largest family of signaling receptors in nature. Their diversity means they play key physiological roles and their dysfunction underlies many pathological conditions, thus they are the focus of many drug design programs due to their primary biological and clinical importance. Our objective is to understand the fundamental mechanisms regulating hormone signaling via GPCRs. Disruption of GPCRs regulation in humans underlies many diseases including reproductive disorders and pregnancy complications, such as polycystic ovarian syndrome, ovarian cancer, infertility, recurrent miscarriage and pre-term births, so a crucial goal is to understand the consequences of these molecular processes on both normal physiological function and in disease. My project aims to elucidate the cellular machinery involved in LH and human chorionic gonadotrophin (hCG) re-ceptor (LH/CG-R) signal regulation and its potential implications in recurrent pregnancy loss. This GPCR is essential in maintaining normal human reproduction by regulating ovulation, spermatogenesis and maintenance of early pregnancy (via actions of the pregnancy hormone hCG). hCG and the LH/CG-R may also play additional roles during pregnancy including regula-tion of embryo implantation and immune responses of the mother, and it is known to be ex-pressed in human endometrial stromal cells (HESCs) which forms part of the lining of the uterus. Preliminary data in the laboratory has indicated that hCG-induced receptor signaling in HESCs is not coupled to the Gαs- pathway (which is the classical pathway of this receptor), but to the Gαi-pathway. Additionally, there may be a change in the constitutive activity of this recep-tor between differentiated and non-differ-entiated HESCs. To our knowledge, no other previous reports have shown a GPCR to change its constitutive activity in the same cell type and this is an exciting novel aspect that will be investigated further in this project. Thus, the overall aim of this project is to unravel the mechanisms underlying the unique signalling and regulatory properties of the LH/CG-R in human endometrium and how such mechanisms could be altered in women with recurrent pregnancy loss.

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P307

Kisspeptin-54 administration stimulates LH pulsatility in women with hypothalamic amenorrhoea

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Introduction
Kisspeptin-54 is a recently identified hormone, which potently stimulates GnRH secretion within the hypothalamus. Women with hypothalamic amenorrhoea (HA, hypogonadotrophic hypogonadism associated with low body weight) have reduced LH pulsatility causing amenorrhea and infertility. We have previously demonstrated that exogenous administration of kisspeptin-54 acutely stimulates gonadotrophin secretion in women with HA. However, it is not known whether exogenous kisspeptin-54 administration can stimulate LH pulsatility.
Methods
A single-blinded, placebo-controlled study was performed. Six participants with HA due to low body weight or exercise (mean BMI 18.3) each attended six study visits. Blood was sampled at 10 min intervals for measurement of LH. Participants received a continuous intravenous infusion of saline (placebo) or kisspeptin-54 (doses 0.01, 0.03, 0.1 or 0.3 nmol/kg per h) for 8 h. LH pulsatility was determined by modified Santen and Bardin analysis.
Results
As expected, LH pulsatility was virtually absent in all participants with HA during saline administration. Administration of kisspeptin-54 significantly increased mean serum LH and the number of LH pulses in a dose-dependent manner. Maximal effects were observed during 0.3 nmol/kg per h kisspeptin-54 infusion, which induced a 12-fold increase in basal LH secretion, and sixfold increase in number of LH pulses (mean LH in IU/l: 1.1, saline; 12.9, 0.3 nmol/kg per h, $P=0.011$; mean number of pulses/8 h: 0.67, saline; 4.2, 0.3 nmol/kg per h, $P=0.003$).
Discussion
We demonstrate for the first time that exogenous kisspeptin-54 temporarily restores LH pulsatility in women with HA, which has important therapeutic implications. Further work will determine if repeated administration of kisspeptin-54 is able to restore fertility in women with deficient endogenous LH pulsatility.

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P308

Effect of ethnicity on the clinical presentations of women with polycystic ovary syndrome: a 20-year retrospective cohort study

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Methodology
Polycystic ovary syndrome (PCOS) has a variety of signs and symptoms compromising different phenotypic presentations. Insulin resistance is a known association of PCOS. Despite the documented effect of ethnicity on insulin resistance, there is little known about the effect of ethnicity on the clinical presentations of PCOS. We compared the clinical presentations of white and South Asian (SA) women with PCOS.
Methodology
Retrospective analysis of a clinical database of all PCOS women attending one UK endocrine unit (1988–2009). Androgen criteria are defined as one of; hirsutism, acne, androgenic alopecia or increased free androgen index. Anovulation criteria are defined as oligo-amenorrhoea or infertility. Ethnicity was self-registered by patients. Only white and SA data have been presented.
Results
Of 2207 patients, 684 (30%) were SA. White women had a higher metabolic risk profile (hypertension, smoking and BMI) and less diabetes, while SA had more androgenic and less anovulatory criteria, were younger at presentation and had more deprived background (Table 1).

Conclusion
There appears to be a significant affect of ethnicity on the clinical and phenotypic presentation of PCOS. Understanding these differences may help tailor appropriate management in defined groups of patients with this condition.

Table 1 Characteristics of women with PCOS (reported as mean (S.D.) or % of that population)

Variable*	White (1523)	SA (684)	Total (2207)	*P* value
Systolic BP	133.2 (15.7)	125.8 (14.7)	130.8 (15.8)	**<0.001**
Diastolic BP	75.06 (11.3)	71.17 (10.2)	73.79 (11.1)	**<0.001**
BMI	31.01 (7.9)	28.27 (6.5)	30.16 (0.19)	**<0.001**
Age	27.3 (7.6)	24.5 (7.3)	26.4 (7.6)	**<0.001**
Deprivation score	18.86 (14.9)	24.9 (14.4)	20.7 (15.0)	**<0.001**
Hypertension-Hx	10.4%	7.6%	9.5%	**0.023**
Smoking-Hx	18.5%	3.7%	13.9%	**<0.001**
Diabetes-Hx	7.2%	9.5%	7.9%	**0.042**
Androgen criteria	85.4%	93.0%	87.7%	**<0.001**
Hirsutism	71.5%	84.6%	75.6%	**<0.001**
Androgenic-alopecia	3.9%	5.6%	4.4%	**<0.01**
Acne	21.5%	20.5%	21.2%	0.306
Anovulation criteria	80.5%	74.4%	78.6%	**<0.01**
Oligomenorrhoea	57.1%	60.8%	58.3%	0.057
Amenorrhoea	18.7%	11.1%	16.4%	**<0.001**
Infertility	16.5%	13.5%	15.5%	**0.039**

*BP, blood pressure; Hx, history.

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P309

A case of persistent Mullerian duct syndrome

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A 45-year-old man who works as a psychotherapist was referred with an incidental finding of a rudimentary uterus and bilateral pelvic gonads on pelvic computerized tomogram and magnetic resonance imaging. This was performed for investigation of abdominal pain which has since settled. As far as he is aware he was born without complications and developed normally through childhood. He progressed through puberty uneventfully with voice changes by 13 years and shaving by 16 years. He was investigated at 20 years for undescended testes confirmed on ultrasound. Surgical exploration of the abdomen revealed no testes and testicular implants were inserted at that time. He has one child with his wife through IVF with donated sperm. He reported good libido, no erectile dysfunction and regular shaving.

On examination he was phenotypically male. Morning biochemistry: testosterone low at 8.6 nmol/l (10.5–30), FSH 39 U/l (1.5–9), LH 12 U/l (1.5–8), oestradiol <50 pmol/l, androstenedione 9.3 nmol/l (3–15), dehydroepiandrosterone sulphate DHEAS 3.5 μmol/l (2–14.5), 17 hydroxyprogesterone 7.3 nmol/l (2–15) and prostate specific antigen 0.8 ng/ml (0–2.5). Karyotyping of 30 lymphocytes demonstrated 46 XY and the sex-determining region Y (SRY) gene was present. He had osteopaenia on bone density scanning. A diagnosis of persistent Mullerian duct syndrome was made. He was counselled, started on testosterone replacement and referred to urology for consideration of abdominal re-exploration. The limited literature currently available suggests that the intra abdominal testes have more malignant potential than the Mullerian structures in this syndrome.

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P310

Pubertal induction in males with hypogonadotropic hypogonadism using long-acting intramuscular testosterone undecanoate 1g depot (Nebido)

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Background
Hypogonadotropic hypogonadism in apubertal males is commonly due to constitutional delay; permanent gonadotropin deficiency becomes more likely with older age at presentation, cryptorchidism and non-reproductive defect, e.g. anosmia. All forms of testosterone induce pubertal development, though short-acting IM preparations are associated with extraphysiological excursions of serum testosterone and are increasingly unavailable. Long-acting testosterone undecanoate IM (TU) is widely-used in men due to superior pharmacokinetics, but data relating to induction of puberty are limited. From 2007, patient preference led us to adopt it for pubertal-induction in hypogonadotropic apubertal males aged 17+ years.
Aims
To audit our experience of IM TU for pubertal induction, focusing on i) patient acceptability/tolerability, ii) maintenance of physiological haematocrit and testosterone levels, and iii) clinical progression through puberty.
Patients and methods
$n=7$ patients presenting 2007–2011; 6/7 assumed to have permanent hypogonadism due to age (mean 37.6 years; range 17.3–57.8) and/or clinical features. Longitudinal data recorded for height, BMI, pubertal staging, pre-injection serum testosterone, haemoglobin and haematocrit. TU administered ~3–4-monthly, guided by lab results and clinical assessment.
Results
Mean treatment duration over the first 3–5 TU injections was 0.91 years (range 0.51–1.04); mean injection interval 13.24 weeks (range 7–18). There were no supraphysiologic excursions of serum testosterone, haemoglobin or, haematocrit. No patient experienced any adverse physical or psychological effects, except for male-pattern baldness ($n=1$). All completed pubertal development around a year from treatment-initiation. After 3 years, two older men (age 50.8 and 57.8 years) exhibited major improvement in bone density (23.5 and 40% at L-spine; 26.6 and 46% at hip, respectively).
Conclusions
All seven men completed pubertal development without adverse effects and with excellent adherence to replacement therapy. TU is a safe and effective treatment for the initiation of puberty in males aged 17+ years. Anxieties in respect of inducing puberty in late-presenting apubertal men are largely unfounded.

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P311

White matter changes on magnetic resonance imaging in Klinefelter syndrome

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Introduction
Klinefelter syndrome may involve multiple organ systems. The CNS, magnetic resonance brain imaging (MRI) findings (white matter changes, reduction in ventricular volume and brain size) are under recognised.
We present the case report of a 47-year-old man with schizophrenia who presented with a 4-month history of lethargy, self-neglect and decline in cognitive function. Brain MRI revealed abnormal white matter changes in left frontal and temporal lobes with extension into the basal ganglia. Cerebrospinal fluid analysis revealed a high protein level and normal cytology. Infectious (herpes simplex, toxoplasma, cryptococcus), autoimmune and paraneoplastic causes of encephalitis were excluded. EEG was consistent with encephalopathy; no epileptiform activity was noted. Stereotactic brain biopsy revealed non-specific changes. He initially received empirical treatment for viral encephalitis without clinical improvement. He later responded to treatment with corticosteroids and supportive measures.
Subsequent clinical evaluation revealed long-standing erectile dysfunction, gynaecomastia, abdominal obesity and reduced testicular volume (10 ml). He was found to have hypergonadotrophic hypogonadism (testosterone 6.3 nmol/l (nr 10–32), FSH 18.9 IU/l (nr 1.0–12.0), LH 25.6 IU/l (nr 1.0–12.0). Karyotyping confirmed mosaic Klinefelter syndrome (KS) (47XXY/46XX). Dual-energy X-ray absorptiometry (DEXA) scan revealed osteopenia. Testosterone, calcium

and vitamin D were initiated. Repeat brain MRI scan revealed normal pituitary. The abnormal white matter changes in the frontal lobes persisted with some regression of the temporal lobe changes.
Conclusions
Klinefelter syndrome is under diagnosed and has a higher prevalence in people with schizophrenia. In the presence of unexplained CNS, white matter changes on MRI and clinical features of hypogonadism, karyotyping to exclude Klinefelter syndrome should be considered.

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P312

Metformin treatment of PCOS: St George's Hospital Endocrine Unit Clinical Experience

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Polycystic ovary syndrome (PCOS) is the most common endocrine condition affecting women and is associated with hyperinsulinaemia and hyperandrogenism. Obesity is present in at least 30% of cases and plays a vital role in the development and maintenance of PCOS as well as affecting the severity of the clinical and endocrine features. Significant improvements in symptoms of androgen excess and ovulatory function are seen with even a modest weight loss of 5% in women with PCOS. Metformin is used in PCOS to improve insulin resistance and to achieve weight loss, but there is still controversy as to whether or not metformin aids the latter.
The aim of this study was to determine the impact of metformin treatment on overweight patients with PCOS over the period they attended St George's Hospital Endocrine Unit.
Data was obtained retrospectively on 43 patients with PCOS attending the endocrine clinic at St George's Hospital and prescribed metformin. Patients attended clinic for an average of 5 years. Changes in weight that occurred whilst on metformin treatment and weight changes while not taking the treatment were determined and compared. The average weight of patients when they first attended clinic was 86.5 kg.
The average weight change whilst on metformin was a loss of 2.17 kg. When the patients were not taking metformin, the average weight change was a gain of 3.8 kg. Side effects due to metformin were a major cause of lack of compliance and 50% of the patients took metformin for <6 months.
In conclusion, patients lost significantly more weight whilst taking metformin than when not on the drug, despite its limitations. Non-compliance was high with gastro-intestinal side effects being the main cause.

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Myotonic dystrophy: a rare cause of primary hypogonadism

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Male hypogonadism is usually considered in the presence of classical symptoms like reduced libido, erectile dysfunction and reduced bone mineral density.
We present the case history of a 43-year-old man with learning difficulties who presented with long-standing lethargy. Clinical examination revealed bilateral ptosis, muscle weakness and slow relaxation of handgrip. He had abdominal obesity, pseudo-gynaecomastia, frontal balding, reduced facial, chest, axillary and pubic hair. Testes measured 15mls bilaterally. Investigations revealed hypergonadotrophic hypogonadism ((testosterone 3.0 nmol/l (nr 10–32); FSH 11.3 IU/l (nr 1.0–12.0), LH 11.1 IU/l (nr 1.0–11.0)). Other biochemical investigations: estradiol 193 (<172 pmol/l), 0900 h cortisol 640 nmol/l. Thyroid function tests, prolactin and IGF1 were normal.
Genetic test confirmed the presence of CTG repeat expansion of dystrophia myotonica protein kinase (DMPK) gene consistent with myotonic dystrophy type 1. Electromyography (EMG) revealed frequent myotonic discharges in the distal and proximal limb muscles, consistent with myotonic dystrophy.
Our case report highlights the importance of excluding hypogonadism in the presence of myopathy and specific phenotypic features of congenital muscle disease.

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P314

Abstract withdrawn.

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Steroids

P315

11βHSD1 deficiency increases susceptibility to liver fibrosis by activating hepatic stellate cells

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Background
Liver fibrosis in cirrhosis is characterized by accumulation of extracellular matrix from activated hepatic stellate cells (HSCs). Glucocorticoids (GCs) limit HSC activation *in vitro*. Local GC levels are regulated by 11β-hydroxysteroid dehydrogenase-1 (11βHSD1) which converts inactive GCs (11-dehydrocorticosterone) into active GCs (corticosterone). In this study we hypothesized that 11βHSD1 could potentially inhibit liver fibrosis.
Method
11βHSD1 levels in mouse models of liver injury were investigated. We studied liver fibrotic responses to carbon tetrachloride in mice with global 11βHSD1 deletion (KO) or with administration of a selective murine 11βHSD1 inhibitor, UE2316. Immunohistochemistry, qPCR, western blot and flow cytometry were used to analyse the liver response. Primary mouse HSCs were cultured *in vitro* to investigate the effect of 11βHSD1.
Results
11βHSD1 mRNA and protein levels were decreased concurrently with peak fibrosis and later recovered in liver injury models. Despite lower indices of hepatocyte injury, 11βHSD1 KO mice had exaggerated fibrosis with increased collagen deposition (Col I) and HSC activation (aSMA+). The fibrotic response persisted after injury. An array of profibrotic genes (Col1, aSMA, Tgfb) and genes involved in ECM remodelling (MMP2, MMP9, Timp1) were highly up-regulated in the 11βHSD1 KO mice. In the resolution phase 11βHSD1 KO mice showed an impairment in 'resolving macrophages' populations (decreased F4/80^{int} cd11b^{hi} ly6C^{lo} macrophage ratio). Findings were similar after UE2316 administration during injury. *In vitro* studies showed 11βHSD1 deficient HSCs were more activated than wild type after 8 days in culture and this activation was inhibited by 11-dehydrocorticosterone and corticosterone.
Conclusion
Loss of GCs regenerated within the liver by 11βHSD1 may contribute to unrestrained activation of HSCs following chemical injury and promote liver fibrosis. This contrasts with anti-fibrotic effects of 11βHSD1 deficiency in adipose. Context-specific effects of 11βHSD1 inhibitors on inflammation and repair deserve careful further scrutiny.
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Validation of *CYP11B1* and *CYP11B2* regulation by microRNA-24

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The *CYP11B1* and *CYP11B2* genes encode the enzymes responsible, respectively, for the terminal stages of cortisol and aldosterone biosynthesis, and have been implicated in the development of essential hypertension. Previously, we investigated the role of microRNAs in the regulation of these genes and showed *in vitro* that levels of the adrenally-expressed microRNA-24 (miR-24) inversely correlate with those of *CYP11B1* and *CYP11B2* mRNA, as well as cortisol and aldosterone production. Bioinformatic analysis predicts two putative binding sites for miR-24 in the 3′UTR of *CYP11B1* mRNA and one in *CYP11B2*. The purpose of this study was to ascertain whether observed changes in CYP11B1 and CYP11B2 mRNA levels *in vitro* were due to the direct action of miR-24 at these sites.

Luciferase reporter constructs containing full-length *CYP11B1* and *CYP11B2* 3′UTR sequences were specifically mutated by a single base at the predicted miR-24 binding sites using site-directed mutagenesis. These constructs were then transfected into HeLa cells, either alone or alongside miR-24 inhibitor; luciferase luminescence was measured 48 h post-transfection.

Cells transfected with mutated plasmids yielded significantly higher luminescence compared to non-mutated plasmids ($P<0.01$). Co-transfection of non-mutated plasmids with miR-24 inhibitor also significantly increased luminescence ($P<0.05$), although this effect was eliminated when inhibitor was co-transfected with mutated plasmids ($P=0.24$). Furthermore, combined mutation of both the predicted *CYP11B1* miR-24 sites resulted in greater effect than single mutations at either site.

These results are consistent with canonical miRNA binding and repression, and confirm that miR-24 is capable of regulating *CYP11B1* and *CYP11B2* expression through direct binding of 3′UTR sites on their mRNA. This is the first study to demonstrate directly such regulation of these genes at specific sites, and may have important implications for corticosteroid biosynthesis and its role in the development of hypertension.

Declaration of funding
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Molecular mechanisms underlying the anti-inflammatory properties of 5α-tetrahydrocorticosterone

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Glucocorticoids (GCs) are highly effective anti-inflammatory drugs, however their use is limited by serious side effects. We have previously shown that 5αTHB binds GC receptor (GR) and suppresses inflammation *in vitro* and *in vivo*, without affecting metabolism. Here the underlying molecular mechanisms were explored in cell models of ligand-induced GR phosphorylation, nuclear localisation and gene transcription. Data are mean ± S.E.M. (three experiments); $*P<0.05$ vs vehicle.

Phosphorylation of Ser211GR, influencing nuclear localisation and gene transcription, was assessed by western blot in A549 cells treated (1h) with vehicle, corticosterone (B) (1 μM) or 5αTHB (1–30 μM). Phosphorylation was not induced by 5αTHB alone, in contrast to B (fold induction: 1.4 ± 0.4 (5αTHB, 1 μM), 6.6 ± 1.6* (B).

To determine mobility of ligand-bound GR, localisation of green fluorescent protein tagged-rat GR (GR-GFP) transfected in HEK293 cells was monitored by fluorescence microscopy. Nuclear translocation of GR-GFP by 5αTHB (1 μM) was incomplete (82.7 ± 1.5% reaching the nucleus within 5 h). Conversely, translocation with B (1 μM) was complete within 30 min. A greater proportion (∼3×) of GR-GFP translocated to the nucleus following a sub-maximal dose of B (3 nM; 45 min)) when 5αTHB (1 μM) was present. Nuclear export after steroid washout was observed only with 5αTHB (32.1 ± 6.1% remaining after 24 h). The rate of recovery from nuclear photobleaching (FRAP) suggested that 5αTHB-bound GR-GFP was more mobile in the nucleus than with B (half-life: 5α-THB 3.12 ± 0.38 vs B 4.40 ± 0.42 s; $P<0.05$).

Ligand ability to induce transcription of GR dimer- and multimer-dependent reporter genes (MMTV and PNMT respectively) was tested. Compared to B, 5αTHB (0.1–3 μM; 24 h) was unable to activate either reporter plasmid (fold induction, 5αTHB and B: MMTV-Luc, 1.2 ± 0.04; 16.9 ± 1.1*; PNMT-Luc, 1.6 ± 0.6; 3.1 ± 0.5*).

GR nuclear translocation is slower in presence of 5αTHB, and activation of classical homodimer- and multimer-dependent gene transcription is absent. This may be linked, to an inability to 5αTHB-bound GR to be phosphorylated on key residue Ser211.

Declaration of interest
The co-authors hold a relevant patent for the use of 5aTHB as an anti-inflammatory drug.
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Novel loci for familial autoimmune Addison's disease detected by linkage analysis

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Due to the rarity of autoimmune Addison's disease (AAD), it has proved difficult to gather large case cohorts for genetic studies. Linkage analysis offers a powerful means of identifying genetic susceptibility loci but has never been applied to AAD because of the scarcity of families containing ≥2 affected individuals. We collected DNA from 23 such families to perform the first linkage study in AAD. We genotyped 117 samples (50 cases, 67 controls) from 23 families with ≥2 affected individuals from the UK ($n=12$) and Norway ($n=11$), on the Affymetrix SNP-6.0 array. Data was formatted and quality controlled in PLINK and Merlin was used for linkage analysis. Results were validated by genotyping 65 SNPs (Sequenom) under two of the linkage peaks in 1097 unrelated AAD (693 21-hydroxylase autoantibody positive) and 1117 controls from the UK, Norway and Sweden.

Applying a rare dominant model, three loci on chromosomes 18, 9 and 7 had LOD scores > 2.0. The maximum LOD score of 3.0 was observed within a linkage peak on chromosome 18 (75241668–77950543 kb). Non-parametric analysis revealed one locus on chromosome 6 with maximum LOD score 3.01, in a linkage peak spanning 22375648 – 35968100 kb, which contains the HLA complex. Meta-analysis of the validation study data in the whole cohort revealed association at 3 SNPs underlying the linkage peak on chromosome 18, with maximal association with an intergenic SNP *rs7236339* ($P<0.004$). When those without 21-hydroxylase autoantibodies were excluded, 4 SNPs were associated. 3 of these were intergenic SNPs on chromosome 18, with maximal association at *rs7231100* ($P<0.004$) and one was on chromosome seven, in the *AUTS2* gene (*rs12698902*, $P<0.01$).

This is the first linkage study in AAD and the finding of linkage to the HLA region validates this approach. This study has generated some novel loci, which may cast light on the pathogenesis of AAD.

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Identification of a duplicated P450 side-chain cleavage enzyme (zCyp11a2) defines initiation and maintenance of steroidogenesis in zebrafish

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Zebrafish has emerged as an important vertebrate *in vivo* model to study human disease. Steroidogenesis in zebrafish is not well characterised. Human CYP11A1 (hCYP11A1) catalyses the first step of steroidogenesis, the conversion of cholesterol to pregnenolone. Zebrafish Cyp11a1 (zCyp11a1) is essential during embryogenesis. Published data suggest that zCyp11a1 facilitates steroidogenesis in the interrenal (equivalent to mammalian adrenal), gonad and brain. We identified a duplicated gene, designated as *zCyp11a2*, sharing 85% sequence identity with zCyp11a1.

The aim of this study was to characterise the *zCyp11a* paralogs gene expression pattern and function.

Our RT-PCR data shows that *zCyp11a1* is expressed during early development, from 0 to 22 h post-fertilisation (hpf). Conversely, *zCyp11a2* was only detected after the interrenal is formed (from 32 hpf). Adult gonads expressed both

paralogs. The interrenal and brain expressed only *zCyp11a2*. The *in vivo* zCyp11a function was assessed by knockdown studies using *zCyp11a*-morpholinos. Pregnenolone and cortisol were measured in the developing zCyp11a morphants by liquid chromatography/tandem mass spectrometry (LC/MS/MS). *zCyp11a1* morphants showed an abnormal early development and reduced pregnenolone levels. Transient *zCyp11a2* knockdown impaired *de novo* pregnenolone and cortisol synthesis and resulted in a late phenotype indicative of metabolic abnormalities. zCyp11a enzyme activity was assessed in COS7 cells transiently co-overexpressing adrenodoxin and *zCyp11a* or hCYP11A1. Cells were incubated with 22R-hydroxycholesterol and pregnenolone was measured by LC/MS/MS. zCyp11a2 activity was similar to hCYP11A1, whilst zCyp11a1 activity was significantly reduced.

Our data completely revises our understanding of zebrafish steroidogenesis by defining the roles of the previously described zCyp11a1 and the newly discovered zCyp11a2 enzyme. zCyp11a2 rather than zCyp11a1 is the hCYP11A1 ortholog in zebrafish steroidogenic tissues. Furthermore, we prove that zCyp11a1 activity is significantly reduced but essential during embryogenesis. Importantly, this study proves the value of zebrafish as a comprehensive *in vivo* model in translational research of adrenal and gonadal disease.

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Glucocorticoids enhance insulin sensitivity in human hepatocytes

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Patients with glucocorticoids (GC) excess develop central obesity, insulin resistance and hepatic steatosis in up to 20% of cases. Current dogma suggests that GCs cause insulin resistance in all tissues. However, we have previously demonstrated that GCs induce insulin sensitisation in adipose tissue *in vitro*, whilst causing insulin resistance in skeletal muscle. In rodent hepatocytes, GCs enhance insulin stimulated lipogenesis but studies in human hepatocytes have not been performed and the cellular mechanisms underpinning these observations have not been determined.

Cryopreserved human hepatocytes were purchased from Celsis *in vitro* Technologies (Baltimore, USA) and incubated with variable doses of cortisol (0–1000 nM) for 24 h in the presence and absence of insulin (5 nM). Insulin signalling gene expression levels were quantified by real-time PCR and western blotting was performed to determine total and phospho PKB/akt protein expression levels. *De novo* lipogenesis (DNL) was measured by 1-(14C) acetate incorporation in triglyceride.

GC receptor, IRS1/2, insulin receptor and AKT1/2 were all expressed in primary cultures. Incubation with cortisol alone or in combination with insulin did not significantly alter gene expression levels. However, whilst cortisol treatment did not alter total PKB/akt levels, insulin stimulated phosphorylation of PKB/akt at serine 473 increased following cortisol pre-treatment in a dose dependant manner (1.23-fold (100 nM), 1.68-fold (250 nM), 2.44-fold (1000 nM) vs control $n=4$ $P<0.05$). Increasing doses of cortisol increased insulin stimulated lipogenesis ($43.9\pm12.7\%$ (250 nM), $66.13\pm9.8\%$ (1000 nM) vs control ($23.61\pm10.7\%$), $P<0.05$).

We have demonstrated that in primary human hepatocytes GC treatment enhances insulin signalling through increased serine phosphorylation of PKB/akt and that GCs and insulin can act synergistically to promote lipogenesis. Whilst translation to the clinical setting is crucially important, this mechanism may be fundamental in explaining the interaction between GCs and insulin to drive lipogenesis. Furthermore, this may contribute to the pathogenesis of non-alcoholic fatty liver disease with GC excess.

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The zebrafish ferredoxin orthologue Fdx1b is essential for the redox regulation of interrenal steroidogenesis in larvae and adult fish

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Mitochondrial steroidogenic cytochrome P450 (CYP) enzymes, such as P450 side-chain cleavage, rely on electron transfer from the redox partner ferredoxin (FDX1) for catalytic activity. Previous *in vitro* data suggest these cofactors are key regulators of CYP enzyme activity. This study aims to establish the role of redox regulation on steroidogenesis using zebrafish as a vertebrate *in vivo* model. In contrast to humans, zebrafish have two FDX1 genes, zFdx1 and zFdx1b. Our RT-PCR data shows that zFdx1 is maternally expressed and maintained throughout zygotic development. Its paralog, zFdx1b begins expression between 24 and 36 h post-fertilisation (hpf), which mirrors the expression of the main zebrafish P450 side-chain cleavage enzyme, zCyp11a2. Importantly, this time point coincides with the development of the zebrafish interrenal – the counterpart of the mammalian adrenal gland. While zFdx1 is ubiquitously expressed in adult zebrafish, zFdx1b expression is restricted to the main steroidogenic tissues: interrenal, gonads and brain. This suggests zFdx1b is the redox partner of steroidogenic mitochondrial CYP enzymes in the adult zebrafish. The *in vivo* function of the two zFdx paralogs was characterised using a transient morpholinos knockdown approach. Consistent with the expression data, knockdown of zFdx1 shows early morphological abnormalities during zebrafish development. Alternatively, zFdx1b morphants develop late onset metabolic abnormalities, consisting of an enlarged yolk and a delay in the inflation of their swim bladder at 120 hpf. Cortisol was extracted from zFdx1b deficient larvae and measured by liquid chromatography/tandem mass spectrometry. zFdx1b morphants failed to synthesise cortisol, compared to the injected controls.

This study gives *in vivo* insights into the molecular mechanism of mitochondrial redox regulation of steroidogenesis. We demonstrated that zFdx1b is essential for *de novo* steroidogenesis in zebrafish larvae, and that zFdx1 cannot compensate for Fdx1b function. Overall, our data establishes zebrafish as a model to study inborn errors of adrenal steroidogenesis.

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Steroid profile response to angiotensin II and ACTH in normal volunteer under high and low salt conditions

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Introduction

Steroid profiling using liquid chromatography with tandem mass spectroscopy (LC:MS) has a low cost per sample and has the potential for high through-put processing. However, although this technology is becoming more widely used, little is known of the normal ranges of many less well studied steroid compounds as well as their response to a variety of physiological stimulants.

Methods

Volunteers were recruited to a randomised, double blind cross-over study and adhered to a standard salt diet for 3 days with salt loading (slow sodium tabs) or placebo. After 30 min recumbent rest, samples were obtained pre and post ACTH and angiotensin II infusions. Samples were extracted from plasma using Chem Elute cartridges (Varian, CA, USA) and injected into a C-18-A reversed phase HPLC column. Identification and quantification were accomplished by tandem mass spectrometry using Varian 1200L mass spectrometer.

Results

High and standard salt phases were confirmed by 24 h urinary sodium excretion and plasma renin concentrations (PRC). Mean (24 h Na, standard salt 97.1 ± 39.5 mmol, high salt 199.8 ± 64.6 mmol, $P<0.001$; PRC, standard salt phase 19.1 ± 13.9 mIU/l, high salt phase 9.7 ± 5.9 mIU/l, $P<0.001$). Aldosterone and its immediate precursor, 18-hydroxycorticosterone were stimulated by salt restriction and angiotensin II infusion. There was no difference in deoxycorticosterone or 18-hydroxydeoxycorticosterone following angiotensin II infusion. Cortisol, 11-deoxycortisol, corticosterone and cortisone concentrations were reduced following angiotensin II infusion. As expected, ACTH stimulated all measured corticosteroids compounds.

Conclusions

These data suggest that angiotensin II infusion is associated with a reduction in cortisol and its precursor steroid hormones. In addition, aldosterone synthase is likely to catalyse the 18-hydroxylation of corticosterone but not 18-hydroxylation of deoxycorticosterone. Steroid profiling by LC:MS can reveal a more comprehensive picture of the 'steroid-ome' leading to a greater understanding

of the factors controlling the steroid pathway.
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Mechanisms of estrogen receptor transcription in breast cancer

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Estrogen receptor (ER) is the defining feature of luminal breast cancers, where is functions as a transcription factor in response to the ligand estrogen. The traditional view of ER getting recruited to promoters of target genes is too simplistic. The recent discovery of ER-DNA interaction regions from ER+ breast cancer cell lines has revealed that ER rarely associates with promoter regions of target genes and instead associates with enhancer elements significant distances from the target genes. The genomic mapping of ER binding events also revealed the enrichment of DNA motifs for Forkhead factors. The Forkhead protein FOXA1 (HNF3a) was subsequently shown to bind to approximately half of the ER binding events in the genome and was required for ER to maintain interaction with DNA. We have extended on these findings to map ER binding events in primary breast cancers and distant metastases. We find context dependent ER *cis*-regulatory elements (cistromes) that give insight into underlying transcriptional networks. These differential ER binding profiles correlate with clinical response in ER+ breast cancers. We experimentally explore the binding dynamics between drug sensitive and resistant contexts and identify properties that govern ER binding differences. These data suggest that ER–DNA interactions are dynamic and can be modulated by changes in FOXA1. We are currently exploring mechanisms that mediate FOXA1–DNA interactions, in order to better understand ER transcriptional activity in breast cancer biology. This work provides insight into how estrogen mediates its effects in cancer and how hormone dependent cancers function after acquiring resistance to current endocrine therapies.
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Continuous measurement of free cortisol profiles

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In order to understand the significance of circadian and ultradian hormonal rhythms in man, both in health and disease, it is essential to be able to obtain multiple samples over extended periods, especially during the hours of sleep. The human automated blood sampling (HABS) system reported by Henley and colleagues can achieve this but is recommended for use in the setting of a clinical investigation unit which is its major drawback. For many diagnostic and scientific questions, the most meaningful physiological setting to look at homeostatically important hormones is a subject's home setting. We have now developed an alternative approach to measure glucocorticoid hormones using the technique of subcutaneous microdialysis. This has the additional advantage that it measures the level of active free cortisol as opposed to the total cortisol levels measured in whole blood of which approximately 90% is bound to carrier proteins. Since only the free unbound cortisol has access to tissues and their receptors these are the levels that are physiologically important for glucocorticoid signalling. The technique of microdialysis combined with a novel miniaturised sampling system provides the ability to collect multiple samples automatically without the need for venous access. As part of the validation of this technique, serum samples and corresponding microdialysate samples from either the subcutaneous tissue compartment alone or both subcutaneous tissue and intravenous compartment were collected every 10 min from 1000 to 1400 h in healthy male volunteers aged 19–28 years. Preliminary results confirm that free cortisol is detectable using this technique in both the body compartments and that free cortisol levels reflect spontaneous changes in serum total cortisol measured during the study period. Our early results indicate the potential of this system as a unique tool in research and in clinical diagnosis.
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Dual role of TTC5 cofactor in GR-mediated gene expression

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Glucocorticoid receptor (GR) is a ligand-dependent nuclear receptor which regulates the transcription of a wide spectrum of genes that are responsible for vital immunological, metabolic, developmental, and anti-inflammatory functions. GR transcriptional regulatory effects are modulated by co-regulators including the tetratricopeptide 5 (TTC5) which has been shown to stabilize GR and alter its action in response to cellular stress. TTC5 is a stress-responsive activator of p300 and its activities are controlled by the ataxia telangiectasia mutated (ATM) and Chk2 kinases. TTC5 is comprised of six TPRs in addition to four probable nuclear receptor (NR) LXXLL boxes. Here we provide evidence to suggest that in A549 lung cancer cells different LXXLL motifs in TTC5 are required to differentially inhibit GR mediated transcriptional function on the TAT-3 promoter in a hormone-dependent manner, whereas association of GR with TTC5 through the co-regulator's TPR sequences increased GR mediated TAT-3 gene expression. Furthermore, TTC5 and GR sub cellular co-localization occurred in a mode dependent on the presence of hormone as well as the integrity of the LXXLL domains located in the TTC5 N terminus. We conclude that TTC5 plays a dual function in the control of GR mediated gene expression and its sub cellular location depending on the surface of TTC5 utilized to interact with the receptor.
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Identification of a novel *CYP11B1* isoform in human adrenocortical cells

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The final reaction for cortisol production in the adrenal gland is catalysed by the 11β-hydroxylase enzyme, encoded by the *CYP11B1* gene. Variants in this gene have been associated with alterations in cortisol levels, which increase blood pressure. This gene is traditionally thought to consist of 9 exons. However, recent evidence has predicted the existence of at least one alternatively spliced form.
The presence of novel *CYP11B1* mRNA species in the H295R human adrenocortical cell line and non-diseased human adrenal tissue was investigated using RT-PCR, sequencing and western blotting.
Following RT-PCR, a larger band corresponding in size to an alternative form (ALT1) of *CYP11B1* mRNA was observed on agarose gels, in addition to the wild-type (WT) form. Sequencing of the ALT1 band confirmed the presence of an additional exon between exons 2 and 3. *In silico* analysis of the 26 in-frame amino acids encoded by this exon predicts an insertion between alpha helices B' and C of the enzyme. Western blotting using a custom antibody targeted at this insertion produced a band of the predicted size in total H295R cell protein. Control cells expressing only the WT form of *CYP11B1* did not yield this band.
Further *in vitro* studies are required to investigate the effect of this alternative transcript on protein structure and cortisol production. The identification of a novel *CYP11B1* isoform will broaden our understanding of adrenal physiology and its contribution to cortisol synthesis.
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The role of anti-$ACTH_{1–24}$ antibodies in synacthen-related adverse events

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Background
Immune responses to self-peptides should not generally occur. However, four of 12 autoimmune Addison's disease (AAD) patients developed adverse reactions immediately after synacthen injections, following repeated subcutaneous synacthen injections during a clinical trial (RoSA study). We wondered if these adverse effects were due to the production of anti-synacthen ($ACTH_{1–24}$) antibodies.
Methods
We evaluated the presence of serum anti-ACTH binding activity using immunoblotting and ELISA on sera from participants in the RoSA study ($n=12$; baseline and after synacthen exposure), 131 unrelated patients with AAD, 92 patients with Graves' disease (GD), 15 patients with isolated ACTH deficiency and 102 controls without known autoimmune disease. Immunoblotting was performed on polyacrylamide/tricine gels using commercial synacthen and full-length ACTH peptide (both 10 μg/well). ELISA was performed using $ACTH_{1–24}$ (1 μg/ml) immobilised on solid phase.
Results
Bands at ~4 and ~6 kDa, corresponding to $ACTH_{1–24}$ and full-length $ACTH_{1–39}$ peptide respectively, were found in 10/12 (83%) RoSA study immunoblots, including all those who had an adverse reaction to synacthen. This is in contrast with healthy control sera, which showed no binding. The same 10 subjects from the RoSA study also showed high levels of binding to synacthen by ELISA, along with 28 patients with AAD (21% of 131), 13 patients with GD (14% of 92) and one isolated ACTH deficiency patient (7% of 15). All positive patient sera in the ELISA were tested against the synacthen peptide on immunoblotting, and all ($n=41$) showed specific 4kDa binding.
Conclusion
Our study demonstrates that repeated administration of depot synacthen can lead to anti-$ACTH_{1–24}$ autoreactivity. In addition, a significant number of AAD and GD patients also had similar autoreactivity ($P<0.001$). The presence of these antibodies could mediate some of the adverse effects seen in the RoSA study and explain the well-described phenomenon of resistance to chronic ACTH therapy.
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^{19}F-magnetic resonance spectroscopy as a tool to quantify 11β-hydroxysteroid dehydrogenase activity *in vivo*

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Non-invasive methods to measure enzyme activity *in vivo* can provide a useful tool for the development of selective inhibitors. Tissue-specific dysregulation of 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1), a reductase enzyme that amplifies active intracellular glucocorticoid levels, has been shown in obese patients using invasive tools (biopsy, microdialysis and arteriovenous sampling with stable isotope tracers). 11β-HSD1 inhibitors are efficacious in pre-clinical models of obesity, diabetes, atherogenesis and cognitive dysfunction, but unpredictable pharmacodynamics may explain disappointing results in phase 2 trials. We have explored the use of ^{19}F-magnetic resonance spectroscopy (MRS) and identified suitable fluorinated keto tracer substrates for the *in vivo* monitoring of hepatic 11β-HSD1 both in rat and human. The effect of tracer structure (equivalent fluorine atoms per molecule and distance to the keto/hydroxy group), tracer abundance, scanning time and biological matrix was studied using seven Tesla (small animal) and three Tesla (human) MRI scanners. ^{19}F-MRS responses were linearly related to the total amount of equivalent fluorine atoms. Signals from keto and hydroxy forms differing as little as 0.6 ppm could be resolved and measured simultaneously. We have determined *in vitro* LOD_F (limit of detection as absolute fluorine content) of 0.250 μmol in chloroform and 0.625 μmol in blood, using 400 s/spectrum. *In vivo* detection of tracer 2-(phenylsulfonyl)-1-(4-(trifluoromethyl)phenyl)ethanone and its hydroxy metabolite was achieved in rat liver (7T scanner) after very low oral doses of tracer (5-8 mg). However, this tri-fluorinated tracer is not a licensed pharmaceutical, so studies in humans were progressed with monofluorinated dexamethasone. Oral doses of 10–14 mg were used and under these conditions neither substrate nor product could be detected in human liver. We conclude that MRS monitoring of 11β-HSD1 is feasible, but requires novel multi-fluorinated tracers.
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P450 side-chain cleavage enzyme autoantibodies in canine Addison's disease

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Background
Addison's disease (AD) in both humans and dogs is characterised by corticosteroid deficiency requiring lifelong hormone therapy. In humans autoimmune pathogenesis is established; at diagnosis ~90% of patients are 21-hydroxylase (21-OH) autoantibody positive, with antibodies to other adrenal antigens also detected. The pathogenesis of canine AD is less well characterised; autoimmune mechanisms are suspected, with anti-adrenal autoantibodies demonstrated by indirect immunofluorescence.
Hypothesis
Specific adrenal autoantibodies are detectable in canine AD patients.
Methods: Canine orthologs of 21-OH, p450 side-chain cleavage enzyme (P450scc), 3-β-hydroxysteroid dehydrogenase (3-β-HSD) and 17-α-hydroxylase (17-α-OH) were identified, cloned and S^{35} radiolabelled recombinant proteins expressed. A RIA was validated using radiolabelled human 21-OH and sera of known autoantibody status. Sera from AD dogs and controls with no history of an endocrinopathy or immune-mediated disease were then tested to investigate the presence of autoantibodies; cross-reactivity across species was also assessed.
Results
Radiolabelled proteins were of expected size. Human 21-OH autoantibodies did not cross-react with the canine radiolabelled protein. A proportion, 5/20, of canine AD samples showed reactivity to P450scc, these were a German Shepherd Dog, Beagle, Lurcher, Great Dane and Polish Lowland Sheepdog, comprising three females and two males. Human P450scc autoantibody positive sera from a patient with autoimmune polyglandular syndrome type one (APS1) and a patient with APS2 with associated premature ovarian failure (POF) cross-reacted with canine P450scc.
Conclusions
This is the first study showing P450scc autoantibodies in canine AD. This offers interesting comparisons with human disease, in particular with APS1 and POF, both associated with P450scc autoantibodies. These findings help confirm an immune-mediated component to canine AD, a potential model for the human disease, and could offer a new approach for diagnostic testing in dogs.
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Quantitative analysis of canrenone in plasma by triple quadrupole mass spectrometry

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Canrenone is a mineralocorticoid receptor antagonist used as a diuretic agent to treat hypertension. It is the major active metabolite of spironolactone and may be quantified in clinical studies either to ensure compliance or to gain information about pharmacokinetic-pharmacodynamic interactions.
The aim of this study was to develop and validate a sensitive, quantitative assay for the analysis of canrenone in plasma.
HPLC mass spectrometric method development was carried out on a TQ4 Quantum Discovery triple quadrupole mass spectrometer with an Aria CTC HPLC autosampler system. Under positive electrospray ionisation the major ion detected was the protonated molecular ion $(M+H)^+$ *m/z* 341 for canrenone. Alfaxalone (precursor ion *m/z* 333; 1 μg) was used as the internal standard. Under collisional activation, the major fragmentation ions were *m/z* 107.1 and 91.1 for canrenone and *m/z* 297.3 and 315.3 for alfaxalone. The fragmentation ions were used as quantifier and qualifier ions respectively to add specificity to the assay. Optimal separation of the steroids was achieved using ammonium acetate (5 mM)/methanol at (60:40, 0.3 ml/min) on a Waters C18 T3 Atlantis HPLC column (3 μm; 100×2.1 mm) at 25 °C. A gradient rising from 40 to 90% methanol was applied, with a total run time of 8 minutes.
Validation of quantitative parameters was performed using six intra- and inter-assay replicates. Satisfactory recoveries of canrenone and alfaxalone (106.2% (relative standard deviation (RSD) 9.5%) and 102.2% (RSD 4.7%)) were achieved following liquid-liquid extraction of only 25 μl plasma with ethyl acetate (1:10). The limit of detection was 5 ng/ml and the lower limit of quantitation was 15 ng/ml. The assay was linear ($r=0.9936$) over a range of concentrations (5 ng/ml to 5 μg/ml). The assay proved suitable for quantitation of canrenone in a group of patients who had received a 400 mg dose, in whom concentrations in plasma were found to be in the range 5–45 μmol/l.

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Quality of life relates to glucocorticoid treatment regimen, adiposity and insulin resistance in adults with congenital adrenal hyperplasia: UK Congenital adrenal Hyperplasia Adult Study Executive (CaHASE)

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Background
Quality of life (QoL) has been variously reported as normal or impaired in congenital adrenal hyperplasia (CAH) adults. We found impaired QoL in UK CAH adults and now report the relationship between QoL, glucocorticoid treatment and health outcomes in these patients.
Methods
Cross-sectional analysis of 151 CAH adults with 21-hydroxylase deficiency aged 18–69 years in whom QoL (SF-36), glucocorticoid regimen, anthropometric, and metabolic measures were recorded. Relationships were examined between QoL, type of glucocorticoid (hydrocortisone, prednisolone, hydrocortisone plus prednisolone and any regimen with dexamethasone), and dose of glucocorticoid expressed as prednisolone dose equivalent, PreDDefault (<5, 5–7.4, ≥ 7.5 mg/day). Principal components analysis (PCA) was undertaken to identify clusters of associated clinical and biochemical features and the principal component (PC) scores used in regression analysis as predictor of QoL.
Results
There was a difference in QoL according to glucocorticoid treatment regimen for vitality (ANOVA: $F_{3,144}=5.1$, $P=0.002$) and mental health ($F_{3,144}=3.9$, $P=0.011$). In *post hoc* analysis, vitality and mental health z-scores were higher in patients on hydrocortisone monotherapy compared with the other treatment groups ($P<0.05$). QoL did not relate to PreDEq or mutation severity. In PCA, three PCs were identified that explained 61% of the total variance (r^2) in observed variables. Regression analysis demonstrated that PC2, reflecting *adiposity and insulin resistance* (waist circumference, serum triglycerides, HOMA-IR and HDL-cholesterol) related to QoL scores, specifically impaired physical function ($\beta=-0.72$, 95% CI: -1.11 to -0.34, $r^2=19.9\%$, $P<0.001$), bodily pain ($\beta=-0.51$, 95% CI: -0.77 to -0.23, $r^2=21.6\%$, $P<0.001$), general health ($\beta=-0.50$, 95% CI: -0.80 to -0.20, $r^2=16.0\%$, $P=0.001$) and vitality ($\beta=-0.44$, 95% CI: -0.65 to -0.16 $r^2=15.5\%$, $P=0.002$).
Conclusions
Increased adiposity and insulin resistance, and use of prednisolone or dexamethasone, are associated with impaired QoL in adults with CAH. Further studies are justified to establish whether optimising the choice of glucocorticoid treatment and/or weight loss can improve QoL in this disadvantaged patient group.
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Truncal fat distribution is associated with enhanced glucocorticoid excretion, increased 5α-reductase activity and higher insulin resistance independent of BMI in women with polycystic ovary syndrome

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Polycystic ovary syndrome (PCOS) is a clinical triad of anovulation, hyperandrogenism and insulin resistance. Patterns of fat distribution in PCOS may be associated with androgen activation, glucocorticoid metabolism and insulin resistance. Here we analysed the relationship between fat distribution, steroid metabolism and insulin resistance in women with PCOS.
We compared results from 100 PCOS patients (Rotterdam criteria) with 80 sex- and BMI-matched controls. All patients underwent BMI measurement and body composition assessment by dual-energy X-ray absorptiometry (DEXA), fasting glucose and insulin measurement for homeostatic model assessment of insulin resistance (HOMA-IR) and 24-h urine analysis by gas chromatography/mass spectrometry. The latter included calculation of total glucocorticoid excretion (μg/24 h) and markers of 5α-reductase activity (androsterone/etiocholanolone and 5α-THF/THF ratios). Linear regression analysis was used to measure the impact of fat distribution on glucocorticoid excretion, 5α-reductase activity and insulin resistance.
PCOS and control patients were matched for BMI (32.1 ± 7.1 and 32.2 ± 6.2 kg/m^2 respectively). Compared to controls, PCOS women had higher urinary steroid ratios indicative of 5α-reductase activity (An/Et 1.3 ± 0.6 vs 1.0 ± 0.5, $P=0.005$; 5α-THF/THF 0.9 ± 0.5 vs 0.7 ± 0.4, $P=0.004$) and higher total glucocorticoid excretion (9624 ± 4214 vs 8067 ± 4165, $P=0.013$). After adjustment for age and BMI, increased truncal fat distribution on DEXA was highly predictive of HOMA-IR, glucocorticoid excretion and 5α-reductase activity. For each percentage increase in truncal fat, HOMA-IR values increased by 7.1% (95% CI, 4.6–9.6, $P<0.001$) and total glucocorticoid metabolites by 2.9% (95% CI, 1.3–4.9, $P<0.001$). Total leg fat was a negative predictor of insulin resistance, with each percentage increase in leg fat associated with a 3.6% reduction in HOMA-IR (95% CI 0.11–6%, $P=0.005$).
Body fat distribution in PCOS is closely associated with steroid metabolism and insulin resistance. Truncal obesity is highly predictive of insulin resistance, glucocorticoid excretion and 5α-reductase activity. Increased leg fat may confer beneficial effects on metabolism in patients with PCOS.

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Revival of adrenal function in established autoimmune Addison's disease

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Despite lifelong glucocorticoid and mineralocorticoid replacement, there is excess morbidity and mortality associated with autoimmune Addison's disease (AAD). Adrenal cortical cells undergo continuous self-renewal from a population of subcapsular progenitor or stem cells, under the influence of ACTH. We aimed

to determine if synthetic ACTH analogue could revive adrenal steroidogenic function and ameliorate AAD.
We performed an open-label trial of synthetic $ACTH_{1–24}$ analogue (synacthen) in adults with established AAD for more than 1 year (NCT 01371526). In phase I, depot synacthen 1 mg was administered s.c. alternate days for 10 weeks. In phase II, participants were then randomised to a further 10 weeks of either a continuous 24 h infusion, or overnight 12 h pulsatile synacthen (both administered at 10 μg/h). Dynamic testing of adrenal function was performed every 5 weeks following medication withdrawal. Twelve subjects (aged 16–65; 11 females), were treated for either 10 ($n=2$) or 20 weeks ($n=10$). One participant withdrew after 5 weeks.
Serum cortisol and aldosterone levels remained under 100 nmol/l in 10 of 12 participants throughout the study. However, two participants both with detectable baseline serum cortisol (219 and 179 nmol/l) achieved peak serum cortisol concentrations >400 nmol/l, after 10 and 29 weeks of synacthen therapy, respectively; allowing withdrawal of replacement medication. These patients (both female, with positive 21-hydroxylase antibodies) had AAD for 8 and 4 years respectively. One of them remains well with improving serum cortisol levels 72 weeks after stopping all treatments. The other participant had a gradual reduction in both serum cortisol and aldosterone concentration, hence steroid therapy was recommenced at week-64.
This is the first study to demonstrate that established AAD may be amenable to a regenerative medicine therapy. We have also shown that AAD is a heterogeneous condition in terms of residual adrenal function, and that adrenal progenitor/stem cells may remain dormant for many years.
Declaration of funding
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P334

Range of urinary steroid metabolite ratios in children undergoing investigation for suspected disorder of steroid synthesis

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Background
Calculation of a urinary steroid metabolite ratio (uSMR) may be a useful method of improving diagnostic yield when investigating disorders of steroid hormone synthesis.
Objective & Hypothesis
To investigate the range of uSMR in children with suspected disorders of steroid hormone synthesis.
Population / Methods
Ten ratios were calculated on steroid metabolite data analysed by GC-MS in urine samples collected between 2008-2010 from 219 children who were undergoing investigations. To obtain reference data, urine samples were also analysed in 89 children with no background of endocrine concerns and who had a urine sample collected at presentation to the hospital with an acute illness.
Results
Of the 89 reference children, 36(40%) were male and median age at time of the test was 3 yrs(range,1 month-11 yrs). Of the 219 endocrine patients, 64(29%) were boys. In 129(59%) cases, a urine sample was collected to investigate early or exaggerated signs of adrenarche. Median age at test was 7.4 yrs(1 day-18 yrs). Median and ranges of two steroid ratios used in the diagnosis of 21-hydroxylase deficiency are demonstrated in the Table.

	<6months	6months – <10yrs	10yrs-18yrs
17HP/(THE+THF+ 5alphaTHF) Reference	0.014 (0.005-0.08)	0.005 (0.00-0.25)	0.025 (0.003-0.08)
boys Affected boys	0.014 (0.005-0.102)	0.008 (0.001-0.37)	0.017 (0.001-0.183)
Reference girls Affected girls	0.11 (0.025-0.005)	0.006 (0.00-0.033)	0.011 (0.003-0.069)
PT/(THE+THF+ 5alphaTHF) Reference	0.026 (0.006-0.089)	0.008 (0.001-0.08)	0.02 (0.003-0.089)
boys Affected boys	0.024 (0.008-0.031)	0.021 (0.00-0.283)	0.108 (0.025-0.482)
Reference girls Affected girls	0.046 (0.003-0.186)	0.027 (0.003-0.103)	0.049 (0.019-0.693)
	0.012 (0.009-0.035)	0.019 (0.006-0.128)	0.065 (0.029-0.223)
	0.039 (0.013-0.187)	0.039 (0.005-0.351)	0.085 (0.044-0.299)

Abbreviations
17HP: 17-hydroxypregnanolone, PT: pregnanetriolone, THE: tetrahydrocortisone, THF: tetrahydrocortisol.
Conclusions
These novel data show that reference ranges for urinary steroid metabolite data need to be age matched. Most children with suspected disorders of steroid synthesis have a ratio which is within the reference range and the identification of outliers will lead to better targeting of genetic analyses.
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P335

Gonadotrophic response to operational deployment in Afghanistan

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Background
Military training has been associated with changes in the hypothalamic–pituitary–testicular axis that are consistent with central hypogonadism (fall in testosterone, LH and FSH concentrations). The effects on the hypothalamic–pituitary–testicular axis of deployment to a combat zone are not known. The aim of this study was to clarify this situation.
Methods
Military personnel were investigated pre-deployment (Pre-) and following 3 months in Afghanistan (Mid-deployment). Body mass, body composition and strength were measured, and androgen, thyroid hormone and leptin concentrations were analysed. Data were evaluated by students T test.
Results
Body mass (kg) decreased between Pre- (83.2 ± 9.2 kg) and Mid-Deployment (79.2 ± 8.2 kg) ($P<0.001$). During this period total testosterone concentration did not change but sex hormone binding globulin (SHBG) increased (30.7 ± 9.7 vs 42.3 ± 14.1 nmol/l; $P<0.001$) contributing to a decrease ($P<0.001$) in calculated free testosterone concentration of between 14.3% (measured by RIA) and 23.3% (by liquid chromatography–mass spectrometry). LH and FSH concentration increased by 14.3% ($P<0.001$) and 4.9% ($P<0.003$), respectively. Androstenedione concentration decreased by 14.5% ($P=0.024$) and leptin and free T_3 decreased by 44% ($P<0.001$) and 5.6% ($P=0.033$) respectively. Physical strength was maintained despite the change in body mass or testosterone concentration over this 3-month period.
Conclusion
Free testosterone concentration decreased significantly during the first half of an operational deployment. There was no evidence to suggest that this is due to stress-induced central hypogonadism. Although the mechanisms for increased SHBG levels are not clear, it may be that a fall in body mass and a reduction in leptin concentration conspire to elevate SHBG and contribute to the decrease in free testosterone concentration.
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P336

Reversal of dilated cardiomyopathy in a patient with Cushing's syndrome after a successful adrenalectomy

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Cushing's syndrome (CS) associated with dilated cardiomyopathy without LVH is rare but important to recognise as treatment of CS can lead to total recovery of heart function.
A 30-year-old previously fit and well Turkish man presented with chest pain and was diagnosed with NSTEMI and CCF. An MRI of the heart and CT coronary angiogram showed normal coronary arteries but a large right adrenal tumour of 11.5 cm with extension into IVC. The transthoracic echocardiogram showed a globally dilated left ventricle with an estimated ejection fraction 20%. There was moderate to severe functional mitral regurgitation. Clinically the patient had gross clinical CS and was hypertensive (BP-170/90).

Further investigations revealed Na-140, K-3.3, cortisol after a 1 mg ODST; 509 nmol/l, 24 h urinary cortisol; 4026 nmol/day (normal range 100–379 nmol/day), ACTH <5 ng/l. Aldosterone, renin, urinary metanephrines, DHEA and androstendione all normal. His heart failure was managed with optimal medical treatment and metyrapone was started. Right adrenalectomy with extraction of intracaval tumour thrombus was performed with evidence of complete excision both on histological assessment and a post operative CT scan. Histology confirmed adrenal cortical carcinoma. Weiss score 5.

1 year post surgery, clinical features of Cushing's have almost resolved. Echocardiogram 7 months after adrenalectomy showed a reduction in LV size and an EF of 40-45%. A recent echocardiogram in Turkey showed EF 56%.

Studies examining the relationship between hypercortisolism and cardiac dysfunction suggest that excess cortisol is contributory to cardiac re-modelling and dilated cardiomyopathy, independent of hypertension. The pathophysiology of cardiac remodeling involves complex mechanisms including activation of neurohormonal factors, alpha adrenergic and renin–angiotensin–aldosterone systems. Experimental models have found that the effects of noradrenaline, angiotensin II, and aldosterone can be heightened by hypercortisolism. The saturation of 11β-HSD2 enzyme resulting in mineralocorticoid receptor activation by cortisol has also been suggested as a possible reason for cardiomyopathy in CS.

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P337

Osteoporosis prophylaxis in medical patients taking corticosteroids

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Background

Oral corticosteroids are a known risk factor for developing osteoporosis and subsequent fracture at higher bone mineral density than post menopausal osteoporosis. Bone loss is thought to be most pronounced in the first 12 weeks of steroid use, and existing guidelines recommend a fracture risk assessment and appropriate osteoporosis prophylaxis with calcium and vitamin D supplements and bisphosphonates. There is also a recognized role for FRAX scoring to help stratify osteoporotic fracture risk in these patients.

Methods

Fifty medical inpatients prescribed oral corticosteroids had their 10-year fracture risk calculated using the fracture risk assessment tool (FRAX). The management of these patients was then compared to the current recommendations.

Results

Twenty patients were categorized as low risk, 17 as intermediate risk and 13 as high risk for fragility fractures. Of those patients at low risk, 55% ($n=11$) had appropriate management, this fell to 12% ($n=2$) in the intermediate group and 15% ($n=2$) in the high risk group. Of the remaining patients, 16 low and intermediate risk patients were receiving treatment unnecessarily or before adequate investigation, seven intermediate risk patients were under-investigated and untreated, and 11 high risk patients were not on adequate treatment.

Conclusions

The risk assessment and management of adult patients taking oral corticosteroids is important to minimize the risk of fragility fractures. Patients are currently not being adequately investigated and treated for osteoporosis. We recommend that patients prescribed oral corticosteroids receive a fracture risk assessment on discharge and are managed appropriately in the community.

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P338

A 'Heavy' price of beauty therapy

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Introduction

Iatrogenic Cushing's syndrome from potent topical steroid use, resulting in suppression of the hypothalamic-pituitary-adrenal axis is well recognised. However, this may not be well acknowledged in amongst a general medical take. We report a case of Cushing's syndrome from long term use of skin whitening cream and topical steroids, highlighting the importance of detailed history taking.

Case history

A 49-year-old Nigerian female was admitted on an acute medical take with abdominal pain and osmotic symptoms. She was found to have hyperglycaemia with ketoacidosis which was treated with insulin. The medical team noted her to have persistent hypertension and hypokalaemia requiring treatment. Her presentation prompted further endocrine investigations but she was not referred to an endocrinologist at that stage. She denied using exogenous steroid in any form in the following months of outpatient review. A 0900 h cortisol was 120 nmol/l with two random cortisol of 11 nmol/l. But patient was asymptomatic of adrenal insufficiency. CT scanning of the adrenals performed on the suspicion of Conn's disease, showed no focal adenoma. Because of the discordance in her clinical features and biochemistry, she was eventually referred to an endocrinologist.

Clinical examination was consistent with Cushing's syndrome with marked features of facial plethora, central obesity, proximal myopathy and striking purple striae over the chest and abdomen. Her new onset diabetes mellitus and hypertension were associated complications of steroid excess. The clinical picture of Cushing's syndrome with low early morning cortisol raised the likelihood of exogenous steroid use. Her history was re-visited by endocrinology. Long term topical steroid exposure from skin whitening moisturiser and 0.05% Clobetasol cream was identified and stopped.

Conclusion

This case highlights importance of a focussed history, physical examination, correct interpretation of biochemistry and early referral. A detailed drug history including over the counter medications and skin whitening products containing steroids needs to be elicited.

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P339

Spot urine cortisol: creatinine ratio: a useful screening test for patients with Cushing's syndrome

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Introduction

Cushing's syndrome(CS) is associated with high morbidity and mortality which warrants a good screening test that is less laborious. We explored the possibility of using urine spot cortisol:creatinine ratio (UCCR) as a new screening test for Cushing's syndrome.

Aims and objectives

To study the efficacy of UCCR as a screening test for patients with Cushing's syndrome.

To compare UCCR in patients with CS, obese and normal subjects.

Material and Methods:

This was a prospective study conducted over a period of 1 year (February 2011–January 2012). All patients with CS ($n=15$) were recruited. We also included a subset of obese ($n=15$) and normal weight ($n=5$) subjects. All CS subjects underwent measurement of 24 h urinary free cortisol, midnight serum cortisol and plasma ACTH. An UCCR was measured in an early morning spot sample. Using 12.3 nmol/μmol (mean+2S.D.), based on an earlier study in normal subjects, as the cutoff for UCCR, the sensitivity, specificity, positive and negative predictive values were calculated.

Results

Forty per cent of the patients had Cushing's disease, 33% had adrenal adenomas, 20% had ectopic ACTH producing tumours and 7% had adrenal carcinomas. The mean (S.D.) of UCCR (nmol/μmol) in the CS, obese and normal subjects were 7.0 (+2.7) and 3.5 (+2.7) respectively. There was a significant difference in the mean UCCR of patients with and without CS (obese and normal subjects), 36.0 (+24.7) vs 6.13 (+3.0) nmol/μmol ($P<0.001$).

Using 12.3 nmol/μmol as the cutoff for UCCR, the sensitivity, specificity, positive and negative predictive values were 93.7, 100, 100 and 93.3% respectively.

Conclusion:

With a cut off of 12.3 nmol/μmol, UCCR was found to have high sensitivity and specificity. This inexpensive, rapid, non-invasive test can easily be performed. However, it has to be validated in a larger population with Cushing's syndrome.

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P340

Successful use of subcutaneous infusion of cortisol in an adult case of congentital adrenal hyperplasia

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Congenital adrenal hyperplasia (CAH) is a group a rare autosomal recessive disorders characterised by a deficiency on one of the enzymes necessary for cortisol biosynthesis. More than 90% of CAH is caused by mutations or deletions in cytochrome P450 21-hydroxylase gene. Impaired glucocorticoid synthesis results in chronic elevation of ACTH causing adrenal hyperplasia and accumulation of steroid precursors such as 17-hydroxyprogesterone (17-HOP). The main goal in CAH managemengt is to replace deficient steroids in order to prevent adrenal crises and to suppress the abnormal secretion of androgens. In addition to Mineralocorticoid (fludrocortisone), different glucocorticoids can be used i.e. prednisolone, dexamethazone but more commonly hydrocotisome twice or thrice a day is used. However, the adequate and balanced replacement therapy with glucocorticoid is sometimes difficult to obtain. This is because of number of factors such as patient tolerance, adverse effects and drugs pharmacokinetics.
In this case report, we present a 40 years old lady with a long standing history of congenital adrenal hyperplasia, which failed to be controlled with conventional various modalities and doses of oral glucocorticoid. With good compliance on hydrocortisone 15 mg (morning) and 5 mg (late afternoon) her average 17 HOP was high in the moning at 21 nmol/l and during the day ranged between 2.9 and 4.9 nmol/l. Adjusting hydrocortisone doses and timings could not be tolerated because of significant weight gain and anxiety and difficulty sleeping after the eveing dose. Instead, dexamethasone was tried twice with different doses (0.5–4 mg a day), but caused depression and intolerenace. The 17 HOP profile on dexamethasone was again significatly high in the morining at 47–56 nmol/l.
Using a continuous and variable subcutaneous hydrocortisone infusion via an insulin pump, achieved rapid control of her CAH, attained a normal cortisol circadian and 17 HOP profiles and significantly improved her quality of life. Average daily hydrocortisone dose was 12-17.5 mg/day, which produced on average 24-h serum cortisol and 17-hydroxyprogesterone concentrations of 302.08 and <2.3 nmol/ml, respectively.

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P341

An audit of adrenal venous sampling at University College Hospital, London

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Introduction
Adrenal venous sampling (AVS) is the reference standard test to differentiate between unilateral and bilateral adrenal diseases in patients with primary hyperaldosteronism. Current Endocrine society guidelines recommend AVS in all cases of primary hyperaldosteronism where surgery is desirable and practical. However, this procedure is technically challenging and failure rate is high.
Aims
The aim of this audit was to evaluate success rate of adrenal venous sampling at University College Hospital, London in a retrospective analysis.
Results
We audited all 16 AVS procedures performed on 14 patients in the last 3 years. A procedure was considered successful when adequate cannulation of both adrenal veins was demonstrated. We used cortisol gradient across adrenal vein and peripheral vein to establish success of venous cannulation and applied a cut off value of >2. Right adrenal vein cannulation was successful in 8 (50%) procedures. Left adrenal vein cannulation was successful in 12 (75%) procedures. Both adrenal veins were adequately cannulised in 6 (37%) procedures which were deemed successful. No significant procedure related complications were noted. We reviewed outcomes of the six successful cases. Two patients, who had idiopathic hyperaldosteronism were treated medically. Three patients underwent laparoscopic unilateral adrenalectomy. One (33%) of these three patients had complete cure of hypertension while the other two (66%) had significant improvement in blood pressure control. One patient awaits surgery.
Conclusion
This audit shows that success rate for AVS was nearly 40% at University College Hospital in a 3-year period. This is comparable to many centres across Europe although some centres have much higher success rate. Our success rate is likely to improve as the experience of the radiologists grows. In addition, intraprocedural cortisol measurement is being introduced at the centre which will facilitate further improvement.

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P342

An online survey on awareness regarding steroid therapy and sick day rules

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Introduction
Lifelong corticosteroid replacement is the treatment for patients with Addison's disease. Steroid therapy need to be altered in the event of illness such as infection, accident or any other major stress. Patients need to be advised on sick day rules and this information should be reiterated at every time of patient contact.
We conducted an online survey among doctors across all specialties in the Countess of Chester hospital to assess the knowledge of doctors of varying grades on the management of patients on long term steroids.
Results
47.1% respondents were consultants. 94% said they usually enquire how long patients have been on steroids. 100% said they usually enquire the reason for taking steroids. When asked about changes to be made to steroid therapy during times of illness, 53% answered they will double the dose. 44% answered that the management is not different for patients taking steroids for COPD or rheumatoid arthritis compared to Addison's disease.
88% answered they will give steroids intravenously if patients present with gastrointestinal illness and 94% answered they will give steroids intravenously if patients are nil by mouth. When asked about information which should be given to patients who are taking long steroids, only 50% answered correctly. 82.4% answered that this information should be reiterated at every time of patient contact. 94% answered that they would identify a steroid user by a medic alert bracelet.
When asked to identify oral steroid therapy from a list, 100% answered that prednisolone and dexamethasone are oral steroids. 68.8% identified hydrocortisone as an oral steroid and 47% identified fludrocortisone.
Conclusions
Awareness regarding steroid therapy needs to be improved among doctors of all training grades. Focused education need to be given to all trainees to improve awareness so that patients get appropriate and timely advice.

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P343

Antiphospholipid antibody syndrome: adrenal insufficiency

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Antiphospholipid antibody (APLA) syndrome is a rare autoimmune systemic disorder which can occur as primary condition or secondary to connective tissue diseases, most frequently systemic lupus erythematosis. We are presenting a rare case of a lady who developed adrenal insufficiency secondary to adrenal vein thrombosis, as a late sequel to APLA Syndrome.
A 50-year-old lady admitted to the medical ICU with one day's history of severe abdominal pain and vomiting. PMH includes APLA syndrome and recurrent intrauterine deaths. She was not on any regular medications except OCP which was started a month prior to her hospital admission. Investigations revealed serum sodium 134 (135–150) mEq/l, potassium 3.7 (3.5–5.0) mEq/l, cortisol 20.14 (0.49–58.60) μg/dl, APTT 30.6 (26.1–33.3) s, PT 11.7 (11.0–15.0) s, D-dimer 0.2 (0.0–0.3) mg/l. CT scan showed bilateral adrenal gland and retroperitoneal inflammation. In keeping with past history of APLA syndrome, bilateral adrenal vein thrombosis was considered and she was anticoagulated. Three weeks later, she visited the OPD with tiredness, dizziness and an episode of collapse. BP was 110/70 and 120/80 mmHg on sitting and standing respectively. Morning serum cortisol was 5.38 μg/dl. 250 μg ACTH stimulation test revealed baseline and 30 min cortisol 2.98 and 5.49 μg/dl respectively. She was diagnosed with adrenal insufficiency as a delayed complication of adrenal vein thrombosis. She was started on tablet hydrocortisone 10–5–5mg. Recent ACTH Stimulation test did not show improvement in adrenal response. She has been on maintenance dose of hydrocortisone.
Discussion
The characteristic feature of APLA Syndrome is recurrent arterial and venous thrombosis. However, adrenal vein thrombosis and adrenal insufficiency is rarely reported. OCP along with lack of anticoagulation therapy might have precipitated adrenal vein thrombosis. Even though patient did not have adrenal insufficiency acutely, she developed the condition as a delayed complication. Treating physicians should have high index of suspicion regarding rare complications and monitor them regularly so that life threatening consequences can be avoided.

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P344

Feature of acute mineralocorticoid excess from ACTH secreting bronchial tumour

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Introduction
A 77-year-old man with glipizide treated type 2 diabetes presented acutely unwell, with weakness, intermittent breathlessness, and poor glycaemic control (previously well control with HBA1c of 42 mmol/mol). Initial assessment showed body weight of 74.5 kg, mild leg oedema, blood pressure of 190/100 mmHg, expiratory wheeze, oxygen saturation 91% and PO_2 7.8 mmHg on air. Chest XR showed chronic right lower lobe shadowing and a bulky hilum. His plasma glucose was 22.4 mmol/l, potassium 2.4 mmol/l, and sodium of 145 mmol/l. He was treated for heart failure, started on insulin therapy and triaged to endocrinology for hyperglycaemia. Hypokalaemia was refractory to potassium infusion. Further investigations and management: echocardiogram was normal. CT confirmed 5 cm hilar mass with mediastinal lymphadenopathy, small bilateral pleural effusion, consistent with bronchial carcinoma and potential metastasis to both adrenals. Despite lack of Cushing's feature, Ectopic ACTH secretion was suspected. Urgent random serum Cortisol at 1700 h was very high at 5294 nmol/l with high plasma ACTH of 249 ng/l (normal <50), 24 h Urinary Free Cortisol level was massive at 29 211 nmol/24 h (normal 50–300). He deteriorated within 48 h with significant fluid retention, 5.5 kg weight gain and pleural effusion requiring chest drain. Subsequent confirmatory test for Cushing's syndrome was deemed unnecessary. He was started on Metyrapone with dose actively up-titrated (1 g tds in 12 days) against the mean serum cortisol day series achieving mean cortisol of 980 nmol/l. spironolactone therapy initiated. He responded well, features of mineralocorticoid excess largely resolved (6 kg weight loss) and able to undergo bronchoscopy confirming small cell bronchial carcinoma.
Discussion
Rapidly progressing ACTH secreting bronchial tumour will not present with typical feature of Cushing. High index of suspicion of such a potential diagnosis is crucial. Bulky adrenals in this case were likely ACTH driven not metastasis (altering the staging). Metyrapone remained the first line of therapy improving metabolic changes.

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P345

Cranial diabetes insipidus in a patient with previously cured pregnancy associated adrenal Cushing's syndrome

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Cushing's syndrome in pregnancy is rare and is associated with increased fetal and maternal morbidity. It has previously been described in the presence of ectopic LH receptor expression, and has been associated with gestational diabetes and preeclampsia but to our knowledge has never been associated with any other endocrine pathology.
We here report a 34-year-old woman, who presented with hypertension, weight gain, fluid retention and easy bruising at 11 weeks gestation. On assessment she had marked proximal myopathy, thin skin with pale striae, hypertension: 170/110 mmhg and proteinuria.
Pregnancy associated Cushing's Syndrome was confirmed biochemically. Two 24 h urinary cortisol were 11 344 and 9552 nmol/24 h on successive days. Midnight cortisol was elevated at 755 nmol/l with undetectable ACTH level, and low dose dexamethasone suppression test showed no suppression: 853 to 811 nmol/l. Ultrasound scan revealed a viable 12 week foetus and non-contrast MRI scan confirmed the suspicion of a unilateral adrenal adenoma. She underwent uneventful laparoscopic adrenalectomy at 13 weeks, then received hydrocortisone replacement throughout the rest of the pregnancy which was subsequently withdrawn, and delivered a healthy male fetus at 39 weeks.
Two years later the patient has represented with symptoms suggestive of Diabetes Insipidus. Water deprivation testing has confirmed this diagnosis, with normal repeat urinary free cortisols and low dose dexamethasone testing. MRI pituitary is apparently normal, with no evidence of recurrence on the adrenal CT scan. Autoimmune screen, ferritin, hCG, α-fetoprotein, ESR, and angiotensin converting enzyme as well as anterior pituitary profile are all normal.
The patient has responded well to intranasal desmopressin. However, no unifying diagnosis has yet been confirmed and we believe this to be the first case presenting with this combination of diagnoses.

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P346

ACTH independent bilateral macronodular adrenal hyperplasia presenting as subclinical Cushing's syndrome

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Background
Hypercortisol states present a diagnostic conundrum. Other conditions such as cyclical and subclinical Cushings pose additional challenges. We report a complex clinical presentation of thymoma with coexisting subclinical Cushing's.
Case report
A 65-year-old man presented to chest clinic with breathlessness and anterior mediastinal mass on CXR. CT scan demonstrated a 7.5 cm probable thymoma and bilateral adrenal masses of varying sizes up to 5.3 cm. Patient has well controlled hypertension on four antihypertensive. No other features of hypercortisolism.
24 h urinary free cortisol were normal on three occasions as were 24 h urinary metanephrines and 5HIAA. MRI of adrenals showed atypical appearance for adenomas. PET scan demonstrated positive uptake in anterior mediastinal mass and adrenal glands raising suspicion of hormonal correlation between mediastinal and adrenal masses despite initial normal Endocrine results. CT guided biopsy of mediastinal mass confirmed thymoma.
Repeated hormonal assessment showed non suppressible cortisol after overnight dexamethasone and subsequent LDDST and HDDST also resulted in a failure of cortisol suppression with cortisol (nmol/l) 425 and 497 respectively. ACTH was undetectable (<5 ng/l). Patient underwent thymectomy with perioperative steroid cover.
Repeated interval adrenal MRI remained unchanged. Repeat LDDST and HDDST cortisone non-suppression (403 and 350 nmol/l) respectively. ACTH was <5 ng/l. Iodo cholesterol scan demonstrated ACTH independent macronodular adrenal hyperplasia. Then patient will be treated medically for subclinical Cushing's with interval scans.
Conclusion
Bilateral macronodular hyperplasia is a rare cause and it accounts for 1% of adrenal Cushing's. Patient with subclinical Cushing's may have normal urinary free cortisol level. But there is a failure of cortisol supression by low dose dexamethasone.

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P347

A case of hypocalcaemia in ectopic ACTH production

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A 70-year-old female presented with general lethargy and a two day history of painful muscle twitching and paraesthesia in her right hand. No other symptoms were reported. Past medical history included hypertension, hypothyroidism and she had recently been diagnosed with Type 2 diabetes. Examination was unremarkable.
The overall biochemical picture was that of a hypokalaemic alkalosis with hypocalcaemia and hyperglycaemia: Na^+140 mmol/l, K^+2.9 mmol/l, urea 8.9 mmol/l, creatinine 51 μmol/l, corrected Ca^{2+}2.05 mmol/l, albumin 31 g/l, phosphate 0.64 mmol/l, Mg^{2+}0.89 mmol/l, ALT 61 (<40) IU/l, ALP 252 IU/l, random glucose 21.4 mmol/l, HbAlc 66 mmol/mol, PTH 53.2 (1.5–7.6) pmol/l and 2400 h urinary calcium 3.97 mmol/d (2.5–7.50). CXR revealed a mass at the superior aspect of the right hilum. Venous gas showed a metabolic alkalosis (pH 7.51, HCO_3 32.4).
A subsequent CT confirmed a right upper lobe lung tumour with mediastinal nodes and liver metastases (T3, N2, M1b). During her admission serum calcium spontaneously dropped to 1.67 mmol/l and potassium levels to 2.0 mmol/l. Calcium and potassium levels were normalised with aggressive electrolyte replacement. The association of a primary lung tumour, new-onset diabetes, hypertension and hypokalaemia suggested the possibility of ectopic ACTH. A random cortisol was $>$ 4140 nmol/l, and a follow-up 1mg overnight dexamethasone test showed failure of suppression of cortisol >4140 nmol/l with plasma ACTH 1197 (<47) ng/l.
A diagnosis of Cushing's syndrome secondary to ectopic ACTH production was made. Liver biopsy established a diagnosis of metastatic poorly differentiated neuroendocrine carcinoma of small cell type from a lung primary. Unfortunately the patient's condition deteriorated and she was offered palliative care.
This case describes atypical presentation of ectopic Cushing's syndrome complicated by profound hypocalaemia.

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P348

Bilateral enlarging adrenal masses: when can we wait in indeterminate lesions?

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Case history
A 60-year-old male underwent left sided orchidectomy for a Seminoma in November 2005. As a part of work up for the Seminoma, he was noted to have bilateral adrenal masses which raised the possibility of metastasis.
Investigations
CT scan of the abdomen showed 22 mm mass on the right with a Hounsfield units of −31 and a 12 mm mass on the left with Hounsfield units of −1. The radiological phenotype of these masses along with density measurements were consistent with benign adenomas rather then metastasis. Biochemical assessment confirmed endocrinologically inactive masses with normal overnight 1 mg dexamethasone suppression test and two normal 2400 h urine estimations for catecholamines.
Treatment
Post operatively, he has responded well to chemotherapy and there is no evidence of tumour recurrence. Radiological surveillance for adrenal adenomas was carried out by the oncologists together with surveillance for tumour recurrence with plan to refer to endocrine team if there is evidence of enlargement of adrenal masses. Endocrine follow was lost and hence no endocrine surveillance was carried out for 6 years.
CT assessments in 2008 and 2009 showed no change, however in 2012 it was noted that the right adrenal lesion had increased to 3.2 cm and a further endocrine assessment was sought.
He was seen in endocrine clinic recently and remains asymptomatic and now awaits further biochemical assessment given the increasing size of adenoma and review of his serial radiology after the images have been repatriated to our department.
Conclusions and points for discussion.
Biopsy of adrenal mass in the context of previous malignancy, current increasing size and normal endocrine investigations.
Acceptable length of hormonal and radiological surveillance of solitary adrenal lump, discussion of guidelines which are mostly American and their impact on UK healthcare resources if these are followed.

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P349

Hypokalaemia: a happy outcome

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A 55-year-old lady was referred by her GP to the acute medical unit with a 4-week history of fatigue, generalised swelling and weight gain of a stone, together with a potassium 2.8 mmol/l and sodium 146 mmol/l. Her blood pressure was 211/85 mmHg and she was suspected of having Conn's syndrome. Treatment was started with Amlodipine 5 mg daily and Spironolactone 25 mg daily which was increased to 100 mg daily on subsequent follow-up in the acute medical unit. She remained unwell despite correction of her hypokalaemia and blood pressure and an aldosterone of 66 pmol/l ruled out Conn's. She was discussed with the endocrine team who noticed she had some difficulty getting up from her chair and that she looked cushingoid. Although she had never smoked and had no chest symptoms a chest X-ray was requested which showed a 35 mm opacity in the right middle lobe which was confirmed with a CT scan. 24-h urine cortisol was 11208 nmol/l, random cortisol was >1750 nmol/l and ACTH 262 ng/l, compatible with ectopic ACTH syndrome. She was started on Metyrapone 250 mg qds, increasing to 500 mg qds. At bronchoscopy a vascular lesion was seen suggesting a possible bronchial carcinoid. Bronchial washings were unhelpful. A PET scan showed a metabolically active 3.7 cm mass with SUV max of 4.9. There was also mild metabolic activity in both adrenal glands. A right middle lobectomy was performed and histology confirmed a typical carcinoid tumour. Subsequently after a 1 mg overnight dexamethasone suppression test her cortisol was 24 nmol/l and she made a complete recovery no longer requiring antihypertensive medication.

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P350

An interesting adrenal adenoma: is it just androgen producing or coproducing both androgen and cortisol

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Introduction
We describe a case of young girl, who initially presented with androgen producing adrenal adenoma, and post-operatively, the other adrenal gland profoundly cortisol suppressed, raising suspicion, if it was originally co-producing both androgen and cortisol.
Case report
A 29-year-old girl initially presented, with 8 months history of hirsutism and cranial hair loss.
She had a normal menarche and menstrual cycles and conceived two children without difficulty and no suggestion of early virilisation.
Examination showed facial and abdominal hirsutism, but no clinical features of Cushing's syndrome.
Blood tests showed raised testosterone 5.3 nmol/l (normal <1.5 nmol/l), raised androstenedione 18.7 nmol/l (normal <6 nmol/l) and normal DHEAs of 4.9 μmol/l.
Her aldosterone renin ratio was normal at 284 and 1000 h cortisol was 466. CT scan demonstrated a well circumscribed 5 cm right adrenal mass with no concerning features.
She had a laparoscopic left adrenalectomy and the tumour stain positively with calretinin, Melan-A and inhibin, confirming adreno-cortical origin.
However 6 weeks post-operatively she presented with ongoing tiredness and a short synacthen test showed sub optimal cortisol increase from 150 to 339 nmol/l. She was commenced on hydrocortisone and test repeated 6 weeks later, which re-confirmed that the other adrenal gland is still suppressed with cortisol values increasing from 24 to just 70 nmol/l.
Due to profound suppression of the contra lateral adrenal gland, it is possible that the adenoma was co secreting both androgen and cortisol. Interestingly she had an uneventful surgery and immediate post-op period without any steroid cover.
Conclusion
Although clinically she was not Cushingoid, she could have had screening for Cushing's pre-operatively, as she might have developed acute adrenal crisis during the surgery, due to the suppression of the other adrenal and luckily it did not happen in our case.

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P351

An interesting case of adrenal adenoma

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Introduction
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Conclusion
Although clinically she was not Cushingoid, she could have had screening for Cushing's pre-operatively, as she might have developed acute adrenal crisis during the surgery, due to the suppression of the other adrenal and luckily it did not happen in our case.

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Thyroid

P352

Local regulation of T_3 availability in susceptibility to osteoarthritis

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Local regulation of T_3 action in bone and cartilage is a novel mechanism underlying the pathogenesis of osteoarthritis (OA). Accelerated chondrocyte differentiation is a hallmark of OA and T_3 regulates this process. The type 1 and 2 deiodinases (D1, D2) convert the pro-hormone T_4 to the active hormone T_3 whilst D3 inactivates both T_3 and T_4. D1 contributes to circulating T_3 levels and local T_3 availability is determined by the relative activities of D2 and D3 in target cells. Population studies have recently identified DIO2 and DIO3 as OA susceptibility loci, whilst phase three trials of the T_3 analogue Eprotirome were terminated after toxicology studies revealed cartilage destruction. Thus, increased T_3 action in chondrocytes may increase susceptibility to OA. We hypothesised that deletion of the T_4 activating enzymes would confer protection against OA and thus studied knee joints obtained from 16-week-old adult D2KO and D1D2KO mice 5 μm coronal and sagittal sections at 80 μm intervals were stained with Safranin-O Fast-Green and scored according to the Osteoarthritis Research Society International (OARSI) grading scale (0= normal articular cartilage and 6= clefts or erosions extending to the mineralizing front and covering >75% of the articular cartilage). Maximum and standardized OA scores were determined. A maximum score of two was recorded in D2KO, D1D2KO and WT mice (Kruskal–Wallis, NS, $n=3$–5), indicating loss of articular cartilage surface lamina and the presence of superficial clefts. Standardized OA scores also did not differ among genotypes (WT 7.84 (0–13.5); D2KO 2.07 (0–12.8); D1D2KO 8.26 (0–15.2); median (range) total osteoarthritis score/articular surface, Kruskal–Wallis, ns, $n=3$–5).
These data demonstrate that deletion of thyroid hormone activating enzymes does not affect the onset of spontaneous OA and that disease provocation models will be required to determine the role of local T_3 availability in OA pathogenesis.
Declaration of funding
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P353

Low frequency of pendrin autoantibodies detected using a radioligand binding assay in patients with autoimmune thyroid disease

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Context
Pendrin is a transmembrane protein located at the apical end of the thyrocyte where it mediates the efflux of iodide through the thyroid follicular cell. Recently, pendrin was described as a significant antibody target in Japanese patients with Graves' disease or autoimmune hypothyroidism using an immunoblotting assay. However, a subsequent study failed to verify this in autoimmune thyroid disease patients of Tunisian origin.
Objective
The aim of the current study was to evaluate a UK population of patients with autoimmune thyroid disease for the presence of pendrin autoantibodies using a novel radioligand binding assay.
Results
Sera from 71 Graves' disease and 66 autoimmune hypothyroidism patients and 28 healthy controls were evaluated for pendrin autoantibody reactivity in radioligand binding assays. The results indicated that 7/71 (9.9%) Graves' disease and 5/66 (7.6%) autoimmune hypothyroidism patient sera, respectively, were positive for pendrin autoantibodies. Overall, the frequency of pendrin autoantibodies did not differ significantly between the autoimmune thyroid disease patient cohorts and the healthy control group: $P=0.186$ and 0.317 for Graves' disease and autoimmune hypothyroidism patients, respectively.
Conclusion
Pendrin autoantibodies, detected using a novel radioligand binding assay, are not widely prevalent in UK patients with autoimmune thyroid disease, nor do they differ in frequency between Graves' disease and autoimmune hypothyroidism. These autoantibodies are therefore unlikely to be a useful marker for disease diagnosis, although the role that pendrin may play as an autoantigen in the initiation or maintenance of thyroid autoimmunity remains to be established.

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P354

Interferon induced thyrotoxicosis

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A 44-year-old gentleman with history of intravenous drug abuse, was referred for new onset hyperthyroidism. He was diagnosed with hepatitis C a year prior to presentation. Treatment included pegylated-interferon (IFN-α) 100 μg weekly and Ribavirin 1 g daily. He had early viral response at week 4 of treatment with viral load of <30 IU/ml from 24 089 IU/ml. At week 8, patient complained of lethargy. He was tachycardic, but had no goitre or thyroid eye disease. Thyroid function tests (TFTs) revealed a suppressed TSH <0.02 mU/l (NR 0.35–5.0), FT_4 47.4 pmol/l, (NR 11–23), FT_3 12.6 pmol/l (NR 3.9–6.8). He was started on Carbimazole 20 mg OD. Anti TPO were positive at 1606. TSH receptor antibodies were negative and Isotope thyroid scan showed homogenous uptake. His Interferon treatment was stopped at week 18 and Ribavirin was continued. Successful clearance of virus was achieved 6 months post treatment. His thyroid function tests normalized after starting Carbimazole. Then he became hypothyroid, hence carbimazole was stopped and subsequently, thyroxine was commenced. Unfortunately, he was lost to follow up.
An overall mean prevalence of incident thyroid dysfunction of 6.2% on IFN-α treatment has been reported; hypothyroidism occurring more frequently (3.9%) than hyperthyroidism (2.3%). Thyroid dysfunction was subclinical, and spontaneous resolution occurred in almost 60% of patients with or without withdrawal of interferon. Risk factors for developing thyroid dysfunction were female sex and pre-existing autoimmune thyroiditis. IFN-α can lead to induction of thyroid autoantibodies. In one study, ten patients developed thyrotoxicosis; six of them had clinical manifestations consistent with Graves' disease, and three had transient thyrotoxicosis, with progression to hypothyroidism after resolution of thyrotoxicosis.
Thyroid dysfunction, especially thyrotoxicosis, is not infrequently observed in patients receiving interferon therapy for chronic active hepatitis. It is recommended to measure TFTs before starting IFN, during and after it has been discontinued.

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P355

Levothyroxine therapy affects cerebral blood flow and fatigue in subclinical hypothyroidism

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Background
Overt and subclinical hypothyroidisms are associated with altered cerebral blood flow (CBF) which may be reversed with levothyroxine treatment (T4T). Subclinical hypothyroidism (SCH) is associated with fatigue but it is unclear whether fatigue is related to abnormal CBF and whether T4T has a beneficial impact. We therefore studied fatigued SCH patients before and after T4T and euthyroid non-fatigued healthy controls (HC).
Methods
CBF was measured by arterial spin labelling magnetic resonance imaging on a 3T scanner in 20 SCH subjects (age 40.2±12.1, serum thyroid stimulating hormone (TSH) between 4-10 mIU/l and normal serum free thyroxine) at baseline and after 6 months of T4T 1.6 μg/kg per day ($n=17$ due to dropouts), as well as in 20 age and gender matched HCs. Fatigue was measured by fatigue index score (FIS).
Results
In HC, SCH at baseline and post treatment, the TSH (mean±S.D.) was 2.1±0.9, 6.7±1.8 and 1.9±1.0 mIU/l respectively; FIS was 4.3±5.0, 76.6±23.7 and 34.2±35.7 respectively; and the whole grey matter CBF was 46.9±5.8, 48.8±6.9 and

46.7 ± 8.5 ml/100 g per min respectively. CBF in SCH was non-significantly higher than in HC ($P = 0.3$), and showed a significant decrease after T4T ($P = 0.013$). FIS in SCH was significantly higher than in HC ($P < 0.001$), and showed a significant decrease after T4T ($P < 0.001$). At baseline FIS were not correlated with CBF in SCH.
Conclusions
We found in SCH a non-significant increase in CBF, which was significantly reduced by T4T to the level seen in HC. This suggests that increased CBF was secondary to SCH state. We postulate that slight increase in CBF in SCH may be an over-compensatory response to tissue hypothyroidism. Normalisation of increased blood flow velocities in overt hypothyroidism was found by one group before. The observed fatigue was not associated with CBF in SCH, suggesting that CBF is not a marker of fatigue in SCH.
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P356

Thyroid incidentaloma incidence and malignant prevalence in F-18-FDG-PET/CT imaging
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Background
Thyroid incidentaloma (TI) is an unsuspected, asymptomatic thyroid lesion discovered on an imaging study performed for unrelated purposes. Reported incidence during 2-(18)(F) fluoro-2-deoxy-D-glucose positron emission tomography (^{18}F-FDG-PET/CT) varies from 0.2 to 8.9%, with a quoted thyroid malignancy prevalence of 8-64%.
Method
We reviewed all ^{18}F-FDG-PET/CT scans performed in our institution over 52 months (May 2008-August 2012).
Results
Of 7438 patients scanned, there were localised areas of thyroid activity in 94 (1.3%). Thirty-three patients (35.1%) underwent further investigation, of those, 6 (18.2%) had thyroid cancer– four papillary and two follicular carcinomas; plus one metastatic adenocarcinoma (3.0%). 19 patients (29.5%) had benign disease, 14 determined by ultrasound scan (USS) alone, four by USS and fine-needle aspiration and one histologically. Two patients' (6.1%) USS showed the lesion to be extrathyroidal. Three patients (9.1%) had inconclusive investigations while 2 (6.0%) still await evaluation.
Diffuse thyroid uptake was observed in 46 patients (0.01%). Thirteen (28.3%) of these were previously diagnosed with Hashimoto's thyroiditis. Of the 12 patients (26.1%) with thyroid function tests (TFTs) recorded, seven were abnormal (four hypothyroid, two subclinical hypothyroid, one thyrotoxic).
Conclusion
The incidence of thyroid malignancy in our cohort was lower than that shown in recent studies and systematic reviews. 35.1% of TIs in our cohort were further investigated, consistent with other studies.
Whilst prospective studies are required to accurately identify the prevalence of malignancy in TIs, for many of these patients, a diagnosis of thyroid cancer will not alter management or survival, due to the natural history of the condition for which the ^{18}F-FDG-PET/CT was performed. Clear protocols should be developed to help clinicians manage such TI's. Malignancy rates for TI's cannot be inferred accurately from studies with patient selection bias in the investigation of TI's. All patients with diffuse thyroid FDG uptake should have TFTs.
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P357

Vitamin D status in autoimmune hypothyroidism
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Objective
To investigate vitamin D status in patients with autoimmune hypothyroidism.
Methods
The study group consisted of 100 patients with newly diagnosed Hashimato's thyroiditis and 100 subjects as the control group. Parameters of calcium metabolism, thyroid function tests and 25(OH) vitamin D levels were measured.
Results or Case Presentation
Mean age of the study study groups was 33.4 ± 4.8 years with female:male = 72:28. Vitamin D insufficiency/deficiency (25(OH)D < 30 ng/ml) rate was significantly higher in the Hashimoto's group compared with the control subjects (75 vs 20%, $P < 0.0001$). In the Hashimoto group, mean 25(OH) vitamin D levels were significantly lower compared with the control group (12.5 ± 7.0 vs 22.3 ± 7.9 ng/ml, $P < 0.001$). The study group revealed higher Anti TPO levels in patients vitamin D deficiency 25(OH)D < 20 ng/ml than patients with vitamin D insufficiency group (25(OH)D < 30 ng/ml) (650.4 ± 35.4 vs 340.3 ± 65.4 IU/ml, P 0.001). Serum vitamin D level was inversely correlated with the Anti TPO levels ($r = -0.30$, $P = 0.007$).
Discussion
Vitamin D is involved in immune system and, in particular, on T cell-medicated immunity. Vitamin D receptor is profoundly present in the immature immune cells of thymus and the CD8. Low vitamin D level gives rise to a variety of autoimmune disorders including type 1 diabetes, hypothyroidism.
Conclusion
The higher vitamin D deficiency rates besides lower vitamin D levels in the Hashimoto group together with the inverse correlation between vitamin D and AntiTPO suggest that vitamin D deficiency may have a role in the autoimmune process in Hashimato's thyroiditis.
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P358

Prevalence of anti-thyroglobulin antibodies, their prognostic significance and impact on patient care in a cohort of patients with differentiated thyroid cancer
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Background
The measurement of thyroglobulin (Tg) is important in the follow-up of patients with differentiated thyroid cancer (DTC), but interpretation is subject to interference by anti-thyroglobulin antibodies (TgAb). National guidelines recommend measurement of TgAb status but no consensus exists on how surveillance should be adapted in cases of TgAb positivity.
Aims
To evaluate the impact of TgAbs on clinical management, in a single-centre cohort of DTC patients.
Methods
Retrospective analysis of patients receiving radio-iodine ablation at St Bartholomew's Hospital, London, 1/12/05–31/7/11.
Results
236 consecutive patients met inclusion criteria, of whom 161 were followed-up locally (median duration 1023 days). Forty-three patients (27%) required further treatment and eight (5%) died of DTC during the follow-up period.
96 patients (53.1%) had their TgAb status assessed. Six patients (6%) were TgAb positive. All were female and they were more likely to be younger (median age 32.5 vs 44.1years), and have larger tumours (median size 30.8 vs 22.8 mm), with vascular invasion (66.7 vs 24.4%) and lymph node involvement (66.7 vs 24.4%) at diagnosis. All were alive at most recent follow-up; four had evidence of locally recurrent (three) or metastatic disease (one). Three patients required additional radio-iodine therapy and one had a selective neck dissection. Four patients never had a detectable Tg.
Discussion
Even over a short follow-up period, TgAb positive patients had a high prevalence of recurrent or metastatic disease. The decision to embark on further treatment was based upon the presence of radio-iodine avid or palpable disease as Tg levels were falsely reassuring. The presence of TgAb renders Tg an unreliable recurrence marker. Beyond the usual practices of clinical examination and chest radiography, optimal surveillance strategies in this 'high risk' group are unclear. In our cohort we did not observe that sequential measurement of TgAb resulted in any overt amendments to decision-making.
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P359

Factors prompting thyroid function testing in hospitalised patients with thyroid dysfunction: analysis of a large hospital database

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Thyroid dysfunction is common and the clinical presentation of subjects with abnormal thyroid hormone concentrations varies widely. Whilst acute illness may affect the interpretation of thyroid function tests, delaying diagnosis and treatment of thyroid dysfunction may have significant consequences. We set out to determine the likelihood of thyroid function testing and the factors influencing the probability of serum TSH measurement in hospitalised patients with a recorded diagnosis of thyroid dysfunction. Out of 280 000 admissions between January 2007 and December 2011 to our centre we identified 9912 admissions with a diagnosis of hypothyroidism (91.6%) or hyperthyroidism (8.4%) at discharge. 78.3% were female and the mean age was 66.1 ± 0.7 years. 67.5% were admitted as emergency and mean length of stay was 8.5 ± 0.13 days. The main reason for admission was coded according to the ICD10 classification. The primary reason for admission was circulatory diseases in 22.4%, digestive disorders in 14.5%, neoplasms in 13.6% and respiratory conditions in 7.8%. Serum TSH concentrations were measured in 1852 (18.6%). Longer hospital stay (2–4 days: AOR = 2.11 (1.74–2.57), $P < 0.001$; 5–10 days: AOR = 3.56 (2.95–4.30), $P < 0.001$; >10 days: AOR = 10.33 (8.65–21.36), $P < 0.001$ vs 1 day) and emergency admission (AOR = 2.62 (2.25–3.06), $P < 0.001$ vs elective admission) were associated with increased probability of testing. Diagnosis of hyperthyroidism (AOR = 2.53 (2.11–3.03), $P < 0.001$ vs hypothyroidism) and older age (AOR = 1.01 (1.00–1.01) per annual increment, $P = 0.05$) had higher likelihood of serum TSH measurement. Primary diagnoses of neoplasms (AOR = 0.56 (0.45–0.70), $P < 0.001$) or digestive disorders (AOR = 0.67 (0.55–0.81), $P < 0.001$) were associated with reduced odds of thyroid function testing compared with circulatory diseases.

Conclusions

Only one in five subjects with a recorded diagnosis of thyroid dysfunction underwent thyroid function testing. Admission for circulatory causes and emergency reasons were associated with an increased likelihood of testing. A diagnosis of hyperthyroidism, older age and longer hospital stay were independent factors predicting increased probability of serum TSH measurement. Further analysis may identify patient groups who may benefit from thyroid function testing during hospitalisation.

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P360

Homozygous resistance to thyroid hormone: can cardiac complications be prevented?

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Resistance to thyroid hormone (RTH) is usually due to heterozygous mutations in THRB gene with rare cases being homozygous for receptor defects. We describe an RTH case due to a homozygous TRβ mutation (R243Q).

The Proband (male, 8.4 years), was born at term with low birth weight (1.9 kg) to consanguineous parents. He has a prominent nasal bridge, goitre, low body weight (10th centile), recurrent tonsillitis, hyperactivity and has mild hearing impairment. He has a sinus tachycardia of 125 bpm, mitral and tricuspid regurgitation, and reduced ejection fraction for age (EF 55%). NT-proBNP, a marker of cardiac dysfunction, is elevated (298 pg/ml; rr 10–157).

His circulating thyroid hormones (FT_4 > fourfold; FT_3 > eightfold raised) are very elevated, with normal TSH levels. *THRB* gene sequencing showed homozygosity for a nucleotide substitution, corresponding to an arginine to glutamine change at codon 243 (R243Q) in TRβ. Less abnormal thyroid function (FT_4 1.2–1.8-fold, FT_3 1.2–1.9-fold raised) in each parent and three siblings was associated with heterozygosity for the R243Q TRβ mutation.

Goitre, hyperactivity and recurrent infections had also been noted in another sibling. He developed mitral regurgitation and cardiomegaly and died of heart failure at 13 years, despite diuretic therapy. Although his *THRB* mutation status is unknown, elevation of his thyroid hormones (FT_4 > threefold, FT_3 > 13-fold raised, comparable to the Proband suggest homozygous RTH, with cardiac hyperthyroidism contributing to his mortality.

Previous functional studies indicated unique properties of R243Q TRβ, with significantly impaired ligand-dependent dissociation of mutant receptor homodimers bound to DNA and delayed corepressor release. This might account for unexpectedly significant dominant negative inhibition by R243Q mutant TRβ *in vitro*, correlating with our observed severe homozygous RTH phenotype. Controlling cardiac hyperthyroidism with either medical therapy or thyroid ablation to prevent life threatening decompensation will be a therapeutic challenge.

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P361

Factors predicting the development of hypothyroidism after radioactive iodine treatment

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Background

The use of radioactive iodine treatment (RAI) for the definitive treatment of benign hyperthyroid disorders has been well established. This study was conducted to determine the factors predicting the development of hypothyroidism following RAI therapy.

Methods

All patients ($n = 104$, 25 men, 79 women) who had RAI for hyperthyroidism between January 2008 and December 2009 were included. In 82.6% of patients antithyroid medications were used prior to RAI treatment.

Results

All patients were administered fixed dose of RAI (mean ± S.D.; 402 ± 25.4) MBq. The median (IQR) age of patients was 58 years (54–62). Ninety patients had only one dose of RAI where as fourteen patients received the second RAI treatment after at least 6 months after the first dose which rendered them euthroid or hypothyroid. The success rate of RAI treatment (percentage of patients rendered either hypothyroid (64.4%) or euthyroid (22.1%) after 1 year was 86.5% which is comparable to other studies.

Patients became hypothyroid 138 ± 132 (IQR 32–560) days post RAI. The average dose of thyroxine replacement was 116 ± 39 (range 50–200) μg. The median values for T_3 were 8.3, 5 and 4.4 pmol/;; medians for T_4 were 20, 14 and 13 pmol/l; medians for TSH were 0.05, 0.5 and 0.23 mIU/l at diagnosis, before and after treatment respectively.

When Cox regression analysis was used younger people, lower BMI, higher levels of T_3 and T_4 at diagnosis and prior treatment of antithyroid medications increased the chance of developing hypothyroidism subsequently. When Kaplan–Meir curve was plotted the risk of development of hypothyroidism was lower after 18 months of RAI treatment.

Conclusions

Younger age, lower BMI, higher levels of T_3 and T_4 at diagnosis and prior treatment with antithyroid medications were associated with subsequent development of hypothyroidism. The risk of developing hypothyroidism diminishes 18 months after RAI.

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P362

Does closer monitoring of thyroid function post radioiodine reduce the severity of hypothyroidism when first detected?

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We have previously shown that the current guidelines for monitoring thyroid function post radioiodine (RI), may not detect hypothyroidism until it has become severe, in a significant proportion of cases (*J Endocrinol Invest* 2012 **35** 82–86). An alternative more intense follow-up strategy was used whereby patients had TSH and FT_4 measured at 4-week intervals post RI for 6 months. Endocrine specialist nurse-led telephone and nurse-led out-patient clinics were utilized. Data was collected prospectively in 104 patients who received RI for hyperthyroidism. 101 patients were treated with single dose of RI (mean 466 MBq) and three patients received as second dose (mean 550 MBq). Results from this study were compared to our previously published data which used traditional follow-up. We found 75 patients (72%) developed hypothyroidism during the first 6 months of follow-up. Hypothyroidism was detected in 16% of the patients at <4 weeks, 36% at 4–<8 weeks, 28% at 8–<12 weeks, 12% at 12–<16 weeks, 3% at 16–<20 weeks and 5% at 20–<24 weeks. At first detection of hypothyroidism, during intense follow-up, 8/75 patients (11%) had TSH >50 mU/l compared to 44/124

(36%) using our previous data during traditional follow-up ($P<0.001$) and 11/75 patients (15%) had FT_4 <5 pmol/l compared to 34/124 (27%) using our previous data during traditional follow-up ($P<0.03$). Comparing intense follow-up with traditional follow-up; median TSH was 11 mU/l vs 32 mU/l ($P<0.005$) and median free T_4 was 8 vs 7 pmol/l ($P<0.02$) when hypothyroidism was first detected. We conclude that using a more intense follow-up protocol post RI, we can detect hypothyroidism at an earlier and less severe stage.

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Interferon-induced thyroid dysfunction: a case series

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Interferon use for the treatment of chronic hepatitis infection, is associated with the side effect of thyroid dysfunction. This is frequent and can be severe, particularly if not recognised. We performed a retrospective analysis of cases of interferon related thyroid dysfunction referred to our tertiary endocrinology centre. Fourteen cases were identified over the last 8 years. An analysis was carried out of demographic features, presentation, treatment and outcomes.

The mean age was 42.5 years (range 26–52). 57% were female and 43% male. 21% were smokers and 14% had positive family history of thyroid disease. 57% of patients developed hypothyroidism, 21% developed thyroiditis, 14% hyperthyroidism and one patient developed sick euthyroidism. The mean speed of onset of thyroid dysfunction was 12.3 weeks (range 7.7–21 weeks). The most prevalent symptom in patients diagnosed with hyperthyroidism or thyroiditis was sweating (100% of patients), followed by palpitations (80%), increased stool frequency and weight loss (60%). 40% of patients were asymptomatic. Hypothyroid patients presented with weight gain (63%), fatigue (50%) and cold intolerance/poor concentration (25%). Interestingly, 86% of cases had no abnormal physical signs. There were no patients with eye signs.

TPO antibody tests were found to be positive in 36% of patients (mean 620 IU/ml). 64% of patients required treatment with levothyroxine and 29% were managed conservatively. One patient each required treatment with radioactive iodine and carbimazole.

This series demonstrates the breadth of thyroid dysfunction associated with interferon treatment. A female preponderance and a lack of thyroid eye signs was seen, as in previous studies. The mean speed of onset is 12.3 weeks which supports current recommendations to test thyroid function at the start of treatment and every three months. However, the shortest speed of onset was 7.7 weeks, suggesting that earlier testing is advisable if clinical suspicion is high.

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Clinical performance of fine-needle aspiration biopsy of thyroid nodules in a tertiary referral centre

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Fine-needle aspiration biopsy (FNAB) is the tool of choice for evaluating thyroid nodules but there is a significant percentage of insufficient or indeterminate aspirates and falsely reassuring results have been reported in up to 6% of cases. We aimed to examine our experience with FNAB among a large cohort of unselected patients with thyroid nodules.

239 consecutive patients (211 women) underwent FNA of a thyroid nodule between July 2008 and June 2010. Median follow-up 40 months. Data recorded include demographic and biochemical variables as well as Thy grading.

18% were initially diagnosed as Thy 1 (insufficient), 58% as Thy 2 (benign), 19% as Thy 3 (follicular neoplasm), 2% as Thy 4 (suspicious for malignancy) and 3% as Thy 5 (malignant). All patients classified as Thy 4 and 5 had malignancy diagnosed following thyroidectomy. 10 of 45 nodules (22%) classified as Thy3, were ultimately diagnosed as malignant following surgery. Four Thy 2 nodules changed classification following routine repeat FNAB; one patient was reclassified as Thy 5 and was diagnosed with papillary thyroid cancer and 3 were reclassified as Thy 3 – two were ultimately diagnosed with benign disease while one declined lobectomy.

Younger euthyroid patients were more likely to have adverse cytological features. There was no association between gender and histological outcome.

The rate of malignancy among Thy three nodules was high but comparable with reported data. All such nodules should be fully excised. Thy two nodules are very likely to be benign but repeat sampling after 6 months is recommended.

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The challenge of managing refractory amiodarone-induced Graves' disease in resistance to thyroid hormone

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A 42-year-old man with resistance to thyroid hormone (RTH) and a recognised thyroid hormone receptorβ mutation (R383C) mutation, presented with atrial fibrillation (AF) which was resistant to DC cardioversion until initiation of amiodarone therapy.

As expected in RTH, his baseline TFTs were abnormal (FT_4 34.6 pmol/l, TSH 2.27 mU/l), but rose further (FT_4 45 pmol/l, TSH 0.93 mU/l) following commencement of amiodarone. However, shortly thereafter, his thyroid hormones (FT_4 >100 pmol/l, FT_3 22 pmol/l) became very elevated with suppressed TSH (<0.01), positive anti-TSH receptor antibodies (10.4, NR<1) and proptosis, consistent with Graves' disease. Amiodarone was discontinued and high dose carbimazole (60 mg) restored euthyroidism. However, following discontinuation of titrated thionamide therapy at 6 months, his thyrotoxicosis has recurred and he is now controlled with carbimazole. We are considering definitive treatment.

Due to predominance of normal TRα in myocardium, cardiac sensitivity to elevated thyroid hormones is retained in RTH, manifesting as tachycardia (26%) or atrial fibrillation (6%) (1); known association of the R383C TRβ mutation with greater pituitary than peripheral resistance, may have predisposed to AF in this case. As in conventional thyrotoxicosis, AF in RTH is resistant to cardioversion and controlling heart rate with β blockade is usually advised. Although effective, amiodarone therapy can result in thyrotoxicosis (AIT), which can be especially challenging to manage in the context of RTH. Surgical or radioiodine ablation of the thyroid may be required, but the appropriate dose of subsequent hormone replacement is difficult to determine. Thyroxine replacement in conventional dosage is associated with chronically elevated TSH levels, with attendant risk of pituitary thyrotroph hyperplasia or even adenoma formation (2); conversely, supraphysiological T_4 treatment risks cardiac hyperthyroidism and recurrence of AF.

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P366

A rapidly enlarging neck lump and Horner's sign: lessons from a novel case

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Background

Anaplastic carcinoma and primary lymphoma (TL) each constitute <2% of thyroid malignancies and are difficult to distinguish clinically. Both typically present with rapidly enlarging anterior neck masses in the elderly. Both may cause pressure symptoms (e.g. dysphagia, stridor and hoarseness). Differentiation is imperative as their treatment and prognoses differ.

Case report

A 68-year-old man presented with a rapidly enlarging thyroid mass, pressure symptoms and an ipsilateral Horner's syndrome (HS). His past history included primary biliary cirrhosis. Clinical examination revealed a fixed 7 cm thyroid mass with no lymphadenopathy. Bloods revealed thyroid stimulating hormone 15 mIU/l, thyroxine 7.6 mIU/l and lactate dehydrogenase 636 μ/l. Full blood count, smear and serum thyroid antibodies were normal. Indirect laryngoscopy

demonstrated a right cord paresis. Fine needle aspiration (FNA) demonstrated mononuclear and atypical cells.
Ultrasound-guided core biopsy revealed diffuse large B-cell lymphoma. Whole body CT demonstrated a large thyroid mass, tracheal deviation with no evidence of lymphadenopathy. Within 5 days of commencing chemotherapy (rituxumab/cyclophosphamide/doxorubicin/vincristine/ prednisolone; R-CHOP) his neck swelling was impalpable. He received external beam radiotherapy. His hoarse voice resolved within a month. The patient is now almost disease free with no clinical sign's of HS at 1-year follow-up.
Discussion
HS in association with thyroid lymphoma has not been reported in the English literature. TL is associated with Hashimoto's thyroiditis, and other auto-immune disorders such as in our case. TL is almost exclusively B-cell in origin: non-Hodgkins type; 71% (aggressive); or mucosa associate lymphoid tissue (MALT); 28% (indolent). Even though FNA is the accepted first line histological investigation for thyroid masses, core or incisional biopsy may be necessary when FNA is inconclusive. Treatment regimes (typically chemotherapy ± radiotherapy) differ for histological sub-types of lymphomas. 5-year failure-free survival is up to 90% in TL compared with a mean survival of 6 months in anaplastic carcinoma.

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P367

The presence of thyroid peroxidase antibodies in Graves' disease is predictive of disease duration and relapse rates

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TSH receptor antibodies (TRAB) are now routinely measured in patients with Graves' disease (GD) to aid diagnosis. Thyroid peroxidase antibodies (TPO) sometimes co-exist in these patients but not always. Some studies have suggested a functional and prognostic role for these antibodies. However, the phenotypic characteristics of the patient with positive TPO (with or without TRAB) and the influence of TPO on the clinical course of GD are not known.
A retrospective analysis of the health records from 14 patients with diagnosed GD who had both TRAB and TPO measured were identified from an endocrine clinic in East Kent. Data was collected on demographics, family history, duration of therapy, relapse rate and the need for early use of definitive therapy. Patients were divided into TPO + ve and TPO − ve groups. Data is expressed as mean ± s.d. and groups compared using un-paired *t*-testing. A P value of <0.05 was considered significant.
Nine patients were TPO + ve. They were predominantly female (89%) and younger (42.2 ± 19.2 years) compared 40% and 50.0 ± 15.8 years respectively in the TPO − ve group. A positive family history was present in 60% TPO + ve patients but in none of the TPO − ve patients. TPO + ve patients were treated for longer (17.9 ± 2.5 vs 13.2 ± 5.0 months, $P=0.046$), had 30% relapse rate within 12months of discontinuing therapy and 20% needed radioactive iodine therapy (RAI) early. None of the TPO − ve relapsed nor required RAI. TPO + ve patients had higher TRAB levels although not significantly so (17.3 ± 16.14 vs 2.6 ± 1.9 U/l, $P=0.19$).
This study has demonstrated that the presence of TPO in Graves' disease results in a phenotype of patients with a more aggressive disease pattern that takes longer to treat, has a higher relapse rate, mainly females and younger age group compared to those patients without TPO. This finding has practical implications for the management of GD but larger studies will be required to confirm the findings.

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P368

Managing Graves' disease: management involving endocrine nurse led service: experience from DGH

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Graves' disease is an autoimmune condition predominantly affecting middle aged women. It can be difficult to manage and consumes a lot of medical time and resources.
In Torbay Hospital, we introduced endocrine nurse specialist (ENS) follow up service to reduce medical burden. This has shifted the work-load of patient care but on the other hand, has resulted in identification of great need for support and increased nurse time.
We reviewed clinical notes of patients with Graves' disease seen by doctors and later by ENS after definitive treatment.
We identified ($n=54$) patients using ENS database. We observed their management during 2011–12, final outcomes, duration of intervention, consultation sessions by doctors and ENS and compared them. Mean age was 17–82 years (median 51). There were 45 females (83%) and 9 males (16%). 48 (88%) presented for the first time while only 6 (11%) had relapsed. There were 398 consultations in total. 219 (55%) sessions were with medical team, while 169 (43%) were with ENS. Furthermore, ENS also provided support and services via phone calls (56), letters (116) and emails or texts (24). 33 (61%) patients received I131 therapy, 5 (9%) underwent thyroidectomy, 10 (18%) patients came off treatment and remained in remission and 6 (11%) are still actively treated. Duration of treatment lasted between 11 and 108 months (median 24 months). 30% patients required monitoring for more than 36 months after definitive treatment.
In summary, introducing endocrine nurse proved efficient service and identified unrecognised need for patient support. It can result in longer follow-up in service. Telephone and email contact is a good thing but presents challenges in recording of information and clinical governance. We believe and there is potential for a protocol driven IT solution to improve quality and effectiveness in the management of Graves' disease.

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Thyroid nodules, FNA cytology and thyroid cancer in Malta

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Introduction
Thyroid nodules are very common and elucidating the nature of these thyroid nodules is an important task.
Methodology
Patients who had an ultrasound guided fine needle aspiration (FNA) of a thyroid nodule between January 2008 and June 2012 were retrospectively audited and their ultrasonographic and biochemical characteristics where analysed. For those patients who were operated nodule characteristics were correlated with thyroid histology.
Results
397 thyroid aspirates were identified. Using The Betsheda System for Reporting Thyroid Cytopathology (TBSRTC) 59.5% were classified as category II (benign), 15.4% category IV (follicular) 4.8% category V (suspicious for malignancy) and 8.4% category VI (malignant).
Statistical analysis of operated patients ($n=97$) yielded a positive predictive value for malignancy (for those who were classified according to TBSRTC categories V and VI) of 89.5%, a negative predictive value of 86.4%, sensitivity of 81.0% and specificity of 92.7%.
42 patients who were operated had thyroid malignancy, of whom 41 had a papillary carcinoma and 1 patient had a medullary thyroid carcinoma. The mean age at presentation was 48.0 years (S.D. $\pm$12.6 years), the mean largest diameter of the papillary carcinomas was 13.8 mm (S.D. $\pm$8.6 mm) and 48.8% had lymph node involvement. 58.5% of patients with malignant histology had more than 1 focus of malignancy in the thyroid. The mean size of thyroid nodule on ultrasound of these patients was 17.5 mm (S.D. $\pm$9.4 mm), 53.7% had a hypoechoic nodule and 48.8% had microcalcifications. These findings differed from those who had a follicular adenoma on histology, where 13.0% had a hypoechoic nodule on ultrasound and 16.1% had microcalcifications.
Conclusions
These findings further establish that FNA of thyroid nodules is a very important and helpful tool in the management of thyroid nodules. Important characteristics of thyroid cancer are shown including the high rate of multifocality seen in our patient cohort.

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False positive pentagastrin stimulation test in a family with medullary thyroid cancer

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We report a case of a false positive pentagastrin stimulation test in a patient with a positive family history for medullary thyroid cancer (MTC), but normal histology following total thyroidectomy.
An asymptomatic 50-year-old man was referred urgently with an elevated calcitonin level of 19.9 ng/l (reference range $<$11.8), taken following the diagnosis of his brother with MTC which had been discovered on a routine private medical examination. There was no family history of multiple endocrine neoplasia. A pentagastrin stimulation test was performed, which demonstrated an elevated calcitonin at baseline (29.8 ng/l), a peak at 1 min (195 ng/l) and a slow return to baseline, consistent with a positive test for underlying MTC.
Further investigations for urinary catecholamines, chromogranin A and B, PTH, calcium thyroid ultrasound and an MRI neck were all normal. Genetic testing was consented for and the results were pending whilst he was under investigation.
The patient proceeded to a total thyroidectomy despite not having the results of genetic testing. The thyroid and cervical lymph node biopsies demonstrated normal histology, with no evidence of C-cell hyperplasia or malignant change. Genetic analysis confirmed carriage of the RET proto-oncogene mutation in the patient's brother and sister, but our patient did not carry the abnormality. Post-operative repeat calcitonin is awaited.
This case questions of the role of the pentagastrin stimulation test as a diagnostic test for MTC. Our patient had a strong family history of MTC and a raised calcitonin level on screening and was adamant that he wanted to proceed to total thyroidectomy. He does not regret having total thyroidectomy and affirms he would make a similar decision again. A second opinion is awaited on the thyroid histology.

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P371

Factors affecting choice of definitive therapy in patients with relapsed thyrotoxicosis

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The use of anti-thyroid medication is favoured first line therapy in Graves' disease (GD). However, relapse rates are high (up to 50%) and definitive therapies of either surgery or radioactive Iodine therapy (RAI) are often considered following an informed decision. The definitive choice taken depends on several factors considered during the patient-doctor interaction. The aim of this study was thus to determine the influence of such factors.
A cross-sectional, qualitative study using a questionnaire based interview approach was used. 14 participants with relapsed GD were recruited at an endocrinology clinic in East Kent. This involved ranking a series of statements from 'strongly agree' to 'strongly disagree' exploring the effect of relatives, doctors, patient knowledge, co-morbidities and misconceptions around RAI. The results are expressed in percentages.
RAI was favoured by 71% of our subjects, surgery by 7%, with others uncertain of their choice. Only 50% of patients could correctly recall the advantages and disadvantages of surgery vs RAI. 14% of patients confessed being frightened by RAI and expressed concerns over continued radioactivity. All (100%) patients disclosed that their relatives would play no part in their decision making process with 78% willing for their doctor to make that final decision for them. The patients' age (above 65 years – 50%) and presence of co-morbidities (14%) were most likely to influence a patient to choose RAI while surgical patients were likely to be younger (50%) and have no other medical conditions (78%).
This study highlights the need to fully clarify to relapsed GD patients the pros and cons of each definitive therapy. Clinicians can be reassured that misconceptions surrounding RAI are low and that in the majority of cases it is the favoured treatment choice. We conclude that the most influential factors considered by relapsed patients were their age and the presence of co-morbidities.

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Levothyroxine absorption testing: a 5-day (usual dose) test as an alternative to the 1-day (1000 μg) test

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Introduction
Despite being on adequate amounts of levothyroxine ($>$1.6 μg/kg) some patients still exhibit biochemical evidence of inadequate replacement (serum TSH $>$ 4.2 mIU/l $\pm$ free-thyroxine (FT_4) $<$12 pmol/l). We report the use of a 5-day absorption test for assessing levothyroxine absorption in such a patient.
Case
A 35-year-old female with hypothyroidism since 2007 had a serum TSH ranging from 4.48 to 54.9 mIU/l and FT_4 ranging from 5.5 to 8.9 pmol/l, despite being on levothyroxine (150–400 μg/day) for several months. She had iron deficiency anaemia but normal serum vitamin B12, folate, calcium and anti-TTG antibody levels, and normal gastro-endoscopic examination. After an unsuccessful six-week trial of the patient self-administering her levothyroxine tablets in the morning on an empty stomach, we therefore performed a supervised 5-day levothyroxine absorption test using her current dose (150 μg/day).
Methods
The patient attended the endocrine unit every morning (Monday to Friday) for supervised administration of her levothyroxine tablets on an empty stomach. Blood samples for thyroid hormones were taken pre-dose on day-1 and 2 h post-dose on day-5.
Results
The 5 day absorption test improved the thyroid hormone profile in this patient by the fifth day. On day-1 the pre-dose serum TSH was 27.9 mIU/l and the FT_4 was 11.3 pmol/l, while on day-5 the 2-h post-dose TSH was 1.9 mIU/l and the FT_4 was 34.6 pmol/l. These results indicate that continued daily supervised administration of levothyroxine (150 μg/day in this case) would have resulted in significant drug-induced thyrotoxicosis.
Conclusion
The supervised 5-day (usual dose) levothyroxine test is as useful as the 1-day (1000 μg) test for assessing levothyroxine absorption in patients with apparent

malabsorption or pseudomalabsorption of levothyroxine, and may be more appropriate for patients who take less than 500 μg/day of levothyroxine. Furthermore, this can be achieved in an outpatient setting. Further studies are required to validate and standardise the 5-day absorption test.

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Four cases of thyroid carcinoma presenting in childhood: 15 years experience in a National Tertiary Referral Centre

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Thyroid cancer has an annual incidence of 0.2–5 per million children representing 3% of all childhood tumours. We describe the presentation, histopathology and treatment of four patients in a regional paediatric surgical and endocrine unit over the past 15 years.

Three of four cases are female with 1 male. Age of diagnosis ranged from 6 to 15 years. All had an elevated thyroglobulin at presentation with normal thyroid function tests. Three underwent FNA and progressed to total thyroidectomy. Two presented with a simple thyroid lump. One had an incidental finding of a neck mass during work-up for renal transplant. The remaining case presented with a neck swelling treated as an inflammatory mass. She later presented with stridor and hoarseness, necessitating urgent operative intervention. One patient had metastases (pulmonary) at presentation. Histology showed one follicular carcinoma and three papillary carcinoma tumours (one follicular variant). All had post-operative radio-iodine (range 1–10 doses) and are disease free at present. We describe follow-up over a range of 18 months to 14 years.

Thyroid carcinoma is a rare diagnosis in children, with limited studies to guide optimum management. We recommend a high index of suspicion when a child presents with a neck lump. Regular follow-up is important especially through teenage years as compliance with thyroxine treatment can be varied. We report remission in all 4 of our cases, highlighting the potential good clinical outcome in this age group.

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P374

Acute transient thyrotoxicosis following intensity-modulated radiotherapy to the neck

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Radiation to the neck is known to be associated with the later development of hypothyroidism. The possibility of acute radiation-induced thyrotoxicosis is not generally recognised. We report here a case of acute hyperthyroidism shortly after radiotherapy.

A 57-year-old man with poorly differentiated adenocarcinoma of the left parotid underwent parotidectomy with radical neck dissection followed by radiotherapy. He received 65 Gy by intensity modulated radiotherapy in 30 fractions. 16 days after completing radiotherapy he developed a sore neck and palpitations. Thyroid function test showed TSH 0.02 mU/l, fT_4 30.6 pmol/l, fT_3 8.2 pmol/l, indicating thyrotoxicosis. He was treated with carbimazole and propranolol by his GP, and referred for specialist care. There was no past or family history of thyroid disease, and there were no symptoms or signs to suggest Graves' disease. TPO was negative. After 11 days of treatment the fT_4 had fallen to 23.9 pmol/l, and fT_3 was normal at 5.8 pmol/l. Radiation-induced thyroiditis was suspected, and after 18 days of treatment carbimazole was stopped. 27 days after diagnosis a ^{99m}Tc thyroid uptake scan showed almost no uptake, indicating acute thyroiditis. After 11 weeks thyroid function returned to normal (TSH 3.38 mU/l); 4 weeks later he developed hypothyroidism (TSH 9.18 mU/l, fT_4 11.5 pmol/l, TSH later 17.84 mU/l) and was treated with thyroxine.

Therapeutic radiation to the neck is known to increase the risk of thyroid dysfunction, with late hypothyroidism the most common abnormality (20–30% incidence with long-term follow-up). Thyrotoxicosis is also reported, but mostly in people with Hodgkin's disease, where there is a 7–20-fold higher risk of developing Graves' thyrotoxicosis after radiotherapy. Thyrotoxicosis due to radiation-induced thyroiditis is uncommon, with only isolated case reports in the literature. These mostly mention asymptomatic thyroiditis; clinically evident thyrotoxicosis is not generally recognised. In thyrotoxicosis due to acute thyroiditis, thionamide therapy should be avoided. Although screening such patients for late hypothyroidism is widely advocated, the value of assessing for hyperthyroidism early after radiotherapy is not known. Thyrotoxicosis from acute thyroiditis after neck irradiation may occur more commonly than is recognised; a prospective study would clarify this.

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P375

Evaluation of fine needle aspiration and ultrasound in diagnostic assessment of thyroid nodules

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Objective

To evaluate the outcome of fine needle aspiration biopsy (FNAB) of solid thyroid nodules, and the utility of thyroid ultrasound in determining the nature of solid nodules in our practice.

Methods

We reviewed the diagnostic outcome of FNAB of solid thyroid nodules in 93 patients from our dedicated thyroid biopsy clinic. In addition we compared the predictive value of sonographic assessment with FNAB in a subset of patients who had undergone both procedures. Samples were assessed for adequacy in the clinic by a cytology technician.

Results

In 24 male and 69 female patients, median age 53 (range 18–84 years), 91% of FNAB were adequate, yielding Thy 2 (64%), Thy 3 (21%), Thy 4 (2%) and Thy 5 (2%) outcomes respectively. 51 benign colloid nodules, 14 follicular lesions, 2 Hurthle cell, 3 papillary, 1 medullary, and 1 anaplastic carcinoma were identified. 8.6% FNAB were classified as insufficient (Thy1), requiring reaspiration. 20 biopsies were Thy3 and 15 patients underwent surgery with histological outcomes of colloid nodule (10), hurthle cell carcinoma (1), follicular carcinoma (3), Hashimoto's thyroiditis (1). 3 nodules yielded benign cytology on subsequent FNAB, 1 patient is awaiting surgery and 1 patient had lymphoma requiring chemotherapy. 3 of 4 Thy 4/5 FNAB proved to be malignant on histology and 1 was consistent with benign colloid nodule.

Fifty patients (53.7%) underwent thyroid ultrasound together with FNAB, enabling comparison of sonographic characteristics with cytological outcome.

Table 1

	Thy 1	Thy 2	Thy 3	Thy 4
Benign USS appearance $n=34$	5	25	3	1
Suspicious USS appearance $n=16$	1	6	7	1

Conclusion

Thyroid nodule FNAB was highly successful (91%) in a dedicated clinic with cytological and radiological support. However, 65% of Thy 3 lesions had a benign histological outcome, suggesting that further diagnostic modalities to evaluate indeterminate nodules and prevent unnecessary surgery are required. Ultrasound had a positive predictive value of 66% and negative predictive value of 86% confirming its utility in deciding which nodules to biopsy.

Table 1

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Thyroid dermopathy: an extreme variant

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Introduction

Thyroid dermopathy usually takes the form of pre-tibial myxedema, which may rarely be caused by Hashimoto's thyroiditis. We present an extreme variant of pre-tibial myxedema, called Elephantiasis nostras verruca (ENV), in a hypothyroid patient.

Case

A 51-year-old woman presented with a progressively worsening growth on her left leg and reduced mobility, constipation, cold intolerance and severe self-

neglect. She was slow to respond to queries. She was obese and bradycardic with dry skin, patchy scalp alopecia, husky voice and no palpable goitre. There was a large ichthyotic mass affecting the left leg. The lower limbs were lymphedematous, hyperkeratotic and hyperpigmented. The patient was severely hypothyroid (free T_4 <2 pmol/l, TSH 93.9 mU/l) with positive anti-thyroid antibodies. HIV, hepatitis and syphilis screens were negative. A skin biopsy was undertaken. Viable epidermal tissue showed spongiosis and pseudoepitheliomatous hyperplasia. The dermis was oedematous with granulation tissue infiltration, and no neoplasm or infection. The patient was commenced on thyroid hormone replacement, with topical emollients and dressings to prevent super-infection. Our working diagnosis is severe thyroid dermopathy secondary to autoimmune hypothyroidism. She will be followed up jointly in the endocrine and dermatology clinics.

Discussion

Our patient has a rare and severe form of dermopathy known as Elephantiasis nostras verruca, which is the result of progressive lymphedema with a cobblestone-like appearance deforming the skin. There are multiple causes including tumours, obesity, scleroderma, and autoimmune thyroid disease. It is usually associated with Graves thyrotoxicosis, but has been reported with Hashimoto's thyroiditis. Biopsy reveals pseudoepitheliomatous hyperplasia, dilated lymphatic spaces, and chronic inflammation. Prolonged accumulation of protein-rich interstitial fluid induces fibroblast proliferation and increases susceptibility to infection and inflammation. Treatments for ENV are challenging and include conservative measures to reduce stasis, diuretics, and prevention of recurrent infection. Surgical debridement or amputation is needed in recalcitrant cases.

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P377

Too low, too high: is it the Roux-en-Y? Fluctuating thyroid function post obesity surgery

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Hypothyroidism is common condition with a strong female preponderance and a UK prevalence of $\sim$2%. It is normally treated with replacement oral levothyroxine.

Morbid obesity is a costly public health issue with a prevalence in England of $\sim$3% with two-thirds of sufferers being female. Weight loss surgery is increasingly employed as a successful and cost effective intervention for super-morbidly obese patients (BMI >40 kg/m^2) in accordance with NICE guidelines (CG43, 2010).

Our patient is a young woman with primary autoimmune hypothyroidism and co-existent super-morbid obesity (peak BMI $=54.9$ kg/m^2) who underwent laparoscopic proximal Roux-en-Y gastric bypass surgery, subsequently achieving massive weight loss to reach a new stable BMI of 32 kg/m^2. Following surgery, her previously stable dose of levothyroxine of 175 μg ($\sim$0.8–0.9 μg/kg per day) was increased in a stepwise fashion in response to TSH levels indicating under-replacement. Subsequently her dose has fluctuated between 250 and 400 μg daily ($\sim$2.9–4.5 μg/kg per day) with no single dose achieving a stable biochemical euthyroidism. Adherence to treatment was self-reported to be good. The importance of temporally separating levothyroxine and other interacting medications (including iron containing micronutrient supplements given routinely after malabsorptive weight loss surgical procedures) was impressed upon the patient. Heterophile antibodies against those used in the TSH assay were tested for and excluded.

Previous studies of absorption of levothyroxine before and after gastric bypass have not shown there to be any significant worsening of the absorption profile following surgery. Our experience with this patient would suggest that this may not always be the case.

With increasing use of obesity surgery including gastric bypass procedures to combat obesity, problems with dose adjustments to thyroid hormone replacement and other pharmaceuticals with a narrow therapeutic range is likely to become a more common and potentially challenging problem in our routine clinical practice.

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P378

Not all raised T_4 needed treatment!

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Introduction

Thyrotoxicosis is a common condition referred to endocrine clinics. However, not all of them needed treatment with antithyroid medications. We report a case of thyroid hormone resistance due to a novel TSHRβ mutation who has been treated with subtotal thyroidectomy and antithyroid medications due to raised T_4 levels.

Case report

A 38-year-old south Asian woman was seen in orthopaedic clinic for elective right foot scarf osteotomy for hallux valgus deformity. She has a past medical history of asthma and subtotal thyroidectomy at the age of 11. Incidental blood tests have shown raised FT_4 levels of 38 pmol/l, FT_3 9.8 pmol/l and TSH 6.62 mU/l. She was referred to endocrine clinic and carbimazole 20 mg OD was started in view of symptoms of palpitations, hot flushes and headache. At the same time, bloods were send to check for heterophile antibodies for TSH, anterior pituitary profile including alpha sub unit and MRI pituitary was requested. Anterior pituitary profile came back as normal with alpha sub unit <0.3 and MRI pituitary was normal as well. Despite being on carbimazole and some improvement in symptoms, Her FT_4 was still high at 35 pmol/l with TSH raised at 5.34 mU/l. Her bloods were send to check mutations regarding thyroid hormone resistance and she was found to have a novel heterozygous thyroid receptor β mutation at 1009 A$>$C (p. Thr337Pro). Carbimazole was stopped and she underwent a successful surgery for right hallux valgus deformity.

Discussion

Thyroid hormone resistance syndrome is rare and often treated inappropriately with antithyroid medications or thyroidectomy. Raised TSH with raised FT_4 should alert the clinician about possibility of either TSHoma or thyroid hormone resistance syndrome and should be thoroughly investigated before a decision is made to treat the abnormal thyroid function tests.

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P379

A case of metastatic papillary thyroid carcinoma presenting with pleural, pulmonary and bone metastases

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Background

Differentiated thyroid cancers are reported to present with synchronous distant metastases in 1–9% of cases. The most common single sites of synchronous metastases are lung (45%) and bone (39%) with dual site involvement (12%). Other single sites of metastases are rare (4%). Pleural metastases are very unusual, accounting for $<$ 0.6% of cases.

Case

A 55-year-old male smoker presented with cough, weight loss and thoracic back pain. Examination demonstrated a firm 2 cm right thyroid mass with no palpable lymphadenopathy. CT imaging revealed a right sided, 6 cm pulmonary mass, multiple pulmonary nodules, a pleural effusion and likely bone metastases. Both core needle biopsy of the pulmonary mass and a pleural biopsy stained positive for thyroglobulin and TTF1 and negative for CEA and PSA suggesting metastatic papillary thyroid carcinoma. Ultrasound revealed a nodular thyroid, cytology Thy 3a. The patient underwent a total thyroidectomy with right sided level II–IV lymph node dissection, left sided level VI dissection, resection of the lung right lower lobe and a hilar lymph node clearance. Histology revealed conventional type papillary thyroid carcinoma pT4 N1 (6/53) M1. The resected lung lesion demonstrated metastatic papillary thyroid carcinoma with involvement of sub-pleural and septal lymphatics, infiltration of the visceral pleura and metastatic involvement of local lymph nodes. Post-operatively the patient underwent I131 ablation therapy (8000 MBq). Therapeutic uptake was seen in the pleural and skeletal metastases with radiological progression of relatively iodine poor pulmonary metastases. FDG-PET eight weeks post-therapy showed disease response in the pleura with residual active disease at other sites.

Conclusion

We report a patient presenting with metastatic papillary thyroid carcinoma with pleural, pulmonary and bone metastases. Pleural metastases are a rare site of metastatic spread and are associated with a poor prognosis (median 27 months).

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P380

Raised TSH: a diagnostic conundrum!

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Background
Elevated TSH with raised free T_4 (fT_4) presents a diagnostic challenge. Symptomatically they vary across the spectrum of thyroid status. We report cases with these dilemmas.
Case 1
Seventy four year old was referred with 5 years of TSH ranging between 4.9–7.9 mIU/l (0.2–4.0), fT_4 18.5–27 pmol/l (9.0–19) and free T_3 (fT_3) 4.5–12 pmol/l (2.5–5.7). Thyroid peroxidase antibodies (TPOA) were negative. He reported tremor, weight loss and exertional shortness of breath. He had small goitre, no thyroid ophthalmopathy, normal visual fields, BMI (kg/m^2) 22 and atrial fibrillation.
Results were confirmed with a different assay. FSH was 17.4 IU/l (2–12), Prolactin 516 mIU/l (0–500), SHBG 104 nmol/l (4–71) and alpha subunit 0.95 IU/l (0–1). Thyrotropinoma was suspected and thyroid axis was assessed. Partial suppression of TSH to 4.1 with Liothyronine and blunted response to TRH (Baseline TSH 7.4 peaking at 7.67) was seen. MRI pituitary confirmed right sided microadenoma. TSH fell progressively by 42.8% from 8.4 to 4.8 on Octreotide day curve confirming somatostatin analogue sensitivity.
Case 2
A 59-year-old was incidentally found to have deranged TFT in 2008. Though asymptomatic Levothyroxine was commenced. Subsequently TSH was consistently elevated (16.8–32.6) despite normal or raised fT_4 (13.2–23.3) and Thyroxine was steadily increased to 150 μg until referral in 2012.
She was euthyroid, no goitre, BMI 28.8, pulse rate 106 and reported compliance. Pituitary profile and TPOA were unremarkable. Dilution and blocking studies for heterophile antibodies demonstrated interfering antibodies. After removing these, TSH was <0.02. Thyroid replacements were discontinued.
Conclusion
These two cases show contrasting outcomes. Tests need to be interpreted in conjunction with patients' symptoms. High index of suspicion is necessary to investigate normal or raised Thyroid hormones with elevated TSH.

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P381

Bilateral thyroid cysts: an important association not to forget?

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A 64-year-old man was referred with an incidental finding of multiple bilateral thyroid cysts following CT scanning for abdominal pain. He had originally presented with an acute episode of left upper quadrant pain. CT scan of the abdomen demonstrated multiple lesions in the liver compatible with simple cysts. There were also multiple bilateral renal cysts, of which the largest was 10 cm. A small amount of retroperitoneal fluid was seen, probably as a result of a ruptured cyst.
He reviewed by the Urology MDT and it was felt that as his symptoms had resolved and all the cysts appeared simple, no further intervention was necessary. He was referred to our Thyroid Clinic in view of the incidentally discovered bilateral thyroid cysts. He described a family history where his uncle died had suddenly with a history that could be consistent with a ruptured Berry aneurysm. Thyroid function tests were normal. On fine needle aspiration 2 ml fluid was obtained which revealed pigmented macrophages consistent with the diagnosis of a simple thyroid cyst (THY 2).
We considered a diagnosis of adult polycystic kidney disease (APKD) which was confirmed on genetic analysis and he now has regular follow-up with the renal physicians.
This case illustrates the unusual association between multiple bilateral thyroid cystic disease and APKD. APKD is characterised by formation of multiple renal and hepatic cysts. Pancreatic cysts can occur and can rarely cause recurrent pancreatitis. Cystic disease can affect the intracranial blood vessels (Berry aneurysms) and systemic circulation where aortic insufficiency due to aortic root dilatation can occur. Thyroid cysts have been described to be rarely associated with APKD – the only review suggested an incidence of 7%. One should consider this especially in the light of increasing referrals of patients with incidentally found thyroid pathology.

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P382

An unusual association with autoimmune hypothyroidism

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Background
Untreated hypothyroidism may be associated with cutaneous signs including coarse, dry skin and hair loss. Myxoedema (also known as thyroid dermopathy) is usually associated with Graves' thyrotoxicosis but has been reported in patients with hypothyroidism. We describe an unusual skin disorder in a patient with autoimmune hypothyroidism, initially misdiagnosed as myxoedema.
Case
A 41-year-old female with an extensive medical history including autoimmune hypothyroidism, acute myeloid leukaemia, carpal tunnel syndrome and previous patellectomy was referred to clinic due to persistent tiredness and weight gain despite increasing doses of levothyroxine therapy. She was maintained on levothyroxine 125 μg od and her thyroid function tests were consistently within the normal range. Despite this she reported gain in weight of 20 kg over 7 months. She also reported the appearance of multiple, rapidly enlarging, painful, fatty deposits on her legs, which she had been advised were due to hypothyroidism. Clinical examination revealed multiple, large (>10 cm) lipomatous lesions which did not resemble the non-pitting oedema and skin discolouration usually associated with myxoedema. Further investigation resulted in a diagnosis of adiposis dolorosa, known as Dercum's disease. This rare, progressive syndrome of unknown aetiology is characterised by multiple, painful lipomas, obesity, fatigue and mental disturbance. It may also be associated with thyroid dysfunction (usually hypothyroidism), musculoskeletal pain, irritable bowel syndrome and chondromalacia patellae.
Conclusions
It is unusual to see cutaneous signs in patients with hypothyroidism, particularly when receiving adequate levothyroxine replacement. Thyroid dermopathy is usually associated with Graves' disease, thus patients with hypothyroidism who report skin changes as a prominent feature may warrant further examination. Adiposis dolorosa is a rare, progressive skin disorder which usually develops in middle age and is five times more common in women. It may be associated with thyroid dysfunction and may present as persistent fatigue and weight gain despite biochemical euthyroidism.

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P383

Case report: when measured free T_4 and free T_3 may be misleading. Interference with free thyroid hormones measurements on Roche and Siemens platforms

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A 59-year-old female presented with apathy and 6 kg weight gain. Investigations revealed primary hypothyroidism (TSH >100 μIU/ml). Thyroxine (L-T_4) was started and titrated up to 75 μg, o.d., with clinical improvement. Other investigations revealed high titres of anti-thyroid peroxidase and anti-thyroglobulin antibodies. After three months, there was a fall in TSH to 12.74 μIU/ml, however, with unexpectedly high free T_4 (FT_4) - 6.8 ng/ml and free T_3 (FT_3) - 6.7 pg/ml concentrations (reference range (rr): 0.8–1.9 ng/ml and 1.5–4.1 pg/ml (Siemens) respectively). At this stage L-T_4 was stopped, and this was followed by a rapid increase in TSH (to 77.76 μIU/ml), however, FT_4 concentration remained elevated (2.1 ng/ml). On admission to our Department, she was clinically euthyroid on L-T_4, 88 μg, once daily. Investigations on Roche platform confirmed mildly elevated TSH – 5.14 (rr: 0.27–4.2 μIU/ml) with high FT_4 (4.59 (rr: 0.93–1.7 ng/ml)) and FT_3 (4.98 (rr: 2.6–4.4 pg/ml)). Other tests revealed hypoechogenic ultrasound pattern typical for Hashimoto thyroiditis. There was no discrepancy in calculated TSH value following TSH dilution (101% recovery). Concentrations of FT_4 and FT_3 were assessed on the day of discontinuation of L-T_4 and after four days by the means of Abbott Architect I 1000SR platform. These revealed FT_4 and FT_3 concentrations within the reference range (e.g. FT_4 - 1.08 ng/ml (rr: 0.7–1.48) vs 4.59 ng/ml (rr: 0.93–1.7, Roche), FT_3 – 3.70 pg/ml (rr: 1.71–3.71) vs 4.98 (rr: 2.6–4.4, Roche)), confirming assay interference. Concentrations of ferritin and SHBG were normal.
Conclusions
Clinicians must be aware of possible assay interference, including the measurements of FT_4 and FT_3 in the differential diagnosis of abnormal results of thyroid function tests that do not fit the patient clinical presentation.

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P384

Management of thyrotoxic crisis in a brittle asthmatic

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A 35-year lady with Grave's disease and brittle asthma presented to the Endocrinology team with symptoms and signs of thyrotoxic crisis. She was not on any anti-thyroid medication as she had previous suffered a severe rash in response to carbimazole, and profound gastrointestinal disturbance with propylthiouracil. It was suggested that she consider radioiodine to control her condition, but as a single parent with young children she was not prepared to undergo the isolation this would require.
The decision was made to commence Lugol's iodine, and over 6 days her free T_4 improved from 66.2 to 31.7 pmol/l (normal range 9.1–23.8 pmol/l). There was some symptomatic improvement over this time, but due to concerns of rebound hyperthyroidism (Jod - Basedow phenomenon) a total thyroidectomy was performed on day 7.
This procedure was challenging for the surgical team who encountered adhesive tissue planes, but despite this, the parathyroid glands and recurrent laryngeal nerves were identified and preserved, and the operation was uneventful.
The anaesthetic challenge was to overcome the hypermetabolic state and control any catecholamine surge in the perioperative period in a patent with asthma who may experience profound respiratory compromise with beta blocker administration. A midazolam, propofol, vecuronium and remifentanil induction was used with sevoflurane and remifentanil maintenance anaesthesia. In addition, magnesium sulphate was infused to control arrhythmias as a consequence of hyperthyroidism, and hydrocortisone to inhibit further TSH release. It was decided that beta blockers should be trialled in the relatively controlled context of the intubated, ventilated patient in case required postoperatively. A short acting esmolol (6.1 μg/kg per min) infusion was administered without adverse effects.
This case demonstrates how with careful multidisciplinary input and endocrinology work up, a patient experiencing a complicated thyrotoxic storm can be safely managed surgically.

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P385

Audit of use of radioactive iodine in the treatment of thyrotoxicosis at the Bristol royal infirmary (2008–2009)

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Audit was performed to identify outcomes of patients from our department referred for RAI for thyrotoxicosis over 14 month period. Follow-up data of atleast 6 months was required for inclusion. Forty-eight patients were identified; four were excluded due to insufficient follow-up. Diagnoses were: Graves' disease ($n=35$; 79.5%), multinodular disease (MND) ($n=8$; 18.2%) and Amiodarone induced thyrotoxicosis (AIT) ($n=1$; 0.02%).
Graves' disease
Anti-TPO antibodies positive in 15 patients and negative or unmeasured in 21 patients. All antibody unknown or negative patients had clinical features of Graves'. Antithyroid drug therapy (ATDT) of choice was block and replace (B + R) ($n=19$; 56%), followed by Carbimazole titration ($n=12$; 32%). Two patients received Propylthiouracil (PTU). Despite some variation, mean treatment duration was 18 months. One patient required no ATDT. Our RAI standard dose (400 MBq) and given to 27 patients, with higher doses in selected cases (600 MBq $n=6$; 800 MBq $n=2$). Cure (hypothyroidism or euthyroidism) was achieved in 77.8% ($n=21$) of the standard dose group, with 15 requiring thyroid replacement therapy (68%). Two patients required thyroidectomy and 3 had repeat RAI.
MND
Eight cases identified. Carbimazole titration was preferred ATDT ($n=7$) to B + R ($n=1$) and no treatment ($n=1$). Standard dose RAI was received by seven patients and cure achieved in 57.1% ($n=4$; 2 = euthyroid, 2 = hypothyroid).
AIT
This patient commenced Carbimazole titration and RAI 800 MBq was administered after 15 months. The patient was rendered euthyroid.
Despite some variation, our practices followed standard recommendations and although this audit precedes propylthiouracil drug warnings, use was very limited. Overall cure was achieved in 73.5% using standard dose RAI, and is comparable with other centres. Formulating a diagnosis is useful as optimal ATDT management differs between causes.

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P386

Successful pregnancy outcomes with thyroxine treatment in euthyroid women with positive thyroid autoantibodies and recurrent miscarriage

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We present two thyroid antibody positive euthyroid women with history of recurrent miscarriage who had successful pregnancy outcome when treated with levothyroxine.
A 31-year-old Caucasian lady was referred to our endocrine services with a history of three previous miscarriages. She had strongly positive thyroid peroxidise antibodies (TPO) with normal thyroid function tests.
We commenced her on 25 μg of levothyroxine. Within two months, she conceived and the dose was increased to 50 μg daily. She remained on this dose throughout her pregnancy. Thyroid functions were monitored every 6–8 weeks and had remained normal.
Another 28-year-old Caucasian lady was referred pre-conception with a history of two previous miscarriages. She had normal thyroid function with strongly positive TPO antibodies and family history of primary hypothyroidism.
She commenced 50 μg of levothyroxine and conceived 6 weeks later. She remained on the same dose throughout her pregnancy and her thyroid functions were also monitored every 6–8 weeks.
Both ladies had uneventful pregnancies and delivered successfully at 40 weeks gestation. We aimed for thyroid stimulating hormone (TSH) of 1 mU/l. Levothyroxine was stopped after delivery and thyroid functions remained normal 6 weeks later.
There is a strong association between thyroid antibodies and pregnancy loss. Intervention trials with levothyroxine in thyroid antibody positive euthyroid women with recurrent miscarriage are quite limited but have shown a decrease in the miscarriage rate. The data however is insufficient to recommend for or against routine levothyroxine therapy in thyroid antibody positive euthyroid women during pregnancy.
Such patients are at an increased life time risk of developing primary hypothyroidism and require at least annual monitoring of thyroid function tests and again if they plan to conceive to ensure euthyroidism. Management of these patient and the successful outcomes with levothyroxine treatment adds to the limited evidence in this area.

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P387

Carbimazole induced cholestatic hepatitis

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Thyrotoxicosis is a common disorder especially in women. Most of the patients tolerate antithyroid medications very well with very few developing life threatening side effects. We describe a 64 years old gentleman who was diagnosed with hyperthyroidism secondary to Grave's disease (autoimmune). He was treated with Carbimazole 20 mg daily. With in a month, he presented with malaise and reduced oral intake. Laboratory investigations showed acute cholestatic hepatitis with raised alkaline phosphatise (ALP) and alanine transaminase (ALT). His Carbimazole was stopped and was given beta blockers to control his symptoms. His serum Ceruloplasmin, autoimmune screen and iron studies were normal. His viral hepatitis screen for A, B and C was negative and ultrasound scan of his liver was normal The patient's symptoms and laboratory abnormalities resolved with in ten days after withdrawing the offending drug and he was started on Propylthiouracil. His liver functions were normal three months after starting Propylthiouracil. Both Carbimazole and Propylthiouracil can cause liver dysfunction. But since the mechanism of liver damage is different, antithyroid medications can be interchanged without increasing the risk of further liver damage.

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P388

Myxedema Coma: an uncommon presentation of a common thyroid problem

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Myxedema coma has very high mortality and should be suspected in an acutely unwell patient presenting with depressed mental status who is hypothermic, bradycardic and or hypotensive. Myxedema coma may be the first presentation of people with undiagnosed hypothyroidism. Definitive management is with thyroid hormone but supportive measures, identification and treatment of precipitating factors in an appropriately safe environment are vital. There is no consensus about preferred thyroid hormone regimen. Corticosteroid therapy is given until adrenal insufficiency has been excluded. We present a case series in this context highlighting the fact that this condition can still be seen outside textbooks and needs to be considered as a possible differential by general physicians on take.

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