Thyrogen – over a decade of inspiring confidence

- Recommended for ablation by the EANM, ETA, ATA and ESMO1-4
- Preferred for first stimulated follow-up, post ablation by the ETA1
- Licence expanded in 2010 for all WDTC patients (post thyroidectomy) with no distant metastases for pre-therapeutic stimulation in combination with 100 mCi (3.7 GBq) radioiodine5

References:

Thyrogen® thyrotropin alfa 0.9 mg, powder for solution for injection. Please refer to the Summary of Product Characteristics before prescribing. Product composition: Each vial contains 1.1 mg of Thyrogen® following reconstitution with 1.2 ml water for injection. 1.2 ml dose of Thyrogen® contains 0.9 mg of Thyroglobulin alfa. Thyrogen® also contains mannitol, sodium phosphate monobasic, monohydrate, sodium phosphate dibasic, hexahydrate and sodium chloride. Indications: 1. For use with serum thyroglobulin (Tg) testing or without radioiodine imaging for the detection of thyroid remnants and well-differentiated thyroid cancer in post-thyroidectomy patients maintained on hormone suppression therapy (HST). Low-risk patients with well-differentiated thyroid cancer who have undetectable serum Tg levels on HST and nor-thyroid-inconclusive human TSH-stimulated increase of Tg levels, may be followed-up by assaying of TSH-stimulated Tg levels. 2. For pre-therapeutic stimulation in combination with 100 mCi (3.7 GBq) radioiodine for ablation of thyroid tissue remnants in patients who have undergone a near total or total thyroidectomy for well-differentiated thyroid cancer and who do not have evidence of distant metastatic thyroid cancer. Dosage and administration: Two doses of 0.9 mg Thyrogen® should be administered at a 24-hour interval by intramuscular injection in the buttock only. Thyrogen® can only be given to children in exceptional circumstances. No dose adjustment is necessary in the elderly population. Therapy should be supervised by physicians with expertise in thyroid cancer. For radioiodine imaging or ablation, radioiodine administration should be given 24 hours following the final Thyrogen® injection. Diagnostic scanning should be performed 48 to 72 hours following radioiodine administration, whereas post-ablation scanning may be delayed to allow background activity to decline. For diagnostic follow-up serum Tg testing, the serum sample should be obtained 72 hours after the final injection of Thyrogen®. The use of Thyrogen® in patients with reduced liver function does not warrant special considerations. Elimination of Thyrogen is significantly slower in dialysis-dependent end stage renal disease (ESRD) patients, resulting in prolonged elevation of TSH levels for several days after treatment. This may lead to increased risk of hypothyroidism and nausea. There are no studies of alternative dose schedules of Thyrogen® in patients with ESRD to guide dose reduction in this population. In patients with significant renal impairment the activity of radioiodine should be carefully selected. Side effects: The most commonly reported undesirable effects were nausea and headache, occurring in approximately 12% and 7% of patients, respectively. In clinical studies involving 481 patients the following undesirable effects were reported: Very common (≥1/10): nausea, Common (≥1/100 to <1/100): vomiting, diarrhoea, fatigue, asthenia, arthralgia, headache and dizziness. Uncommon (≥1/1000 to <1/100) or unknown frequency: feeling hot, arthralgia, rash, influenza-like illness, jaundice, rigors and back pain, palpitations, dyspnoea, arthralgia, myalgia. Very rare cases of hyperthyroidism or atrial fibrillation have been observed when Thyrogen® 0.9 mg has been given to patients with a partial intact or entire thyroid gland. Manifestations of hypothyroidism have been reported uncommonly in clinical trials and the post-marketing setting, consisting of articular, rash, pruritus and respiratory signs and symptoms. Other uncommon and events of unknown frequency are listed in the SmPC. Precautions: Thyrogen® should not be administered intravenously. When used as an alternative to thyroid hormone withdrawal, the combination of whole body scintigraphy (WBS) and Tg testing after Thyrogen® administration assures the highest sensitivity for detection of thyroid remnants or cancer. If a high index of suspicion for metastatic disease persists, a confirmatory withdrawal WBS and Tg testing should be considered. Because Tg autoantibodies can be present in up to 80% of patients with differentiated thyroid cancer, both Tg and TgAb assays are needed. Careful evaluation of benefit-risk relationships should be assessed for Thyrogen® administration in high risk elderly patients who have heart disease and have not undergone thyroidectomy. Thyrogen® is known to cause a transient but significant rise in serum thyroid hormone concentration when given to patients who have substantial thyroid tissue still in situ. Therefore, careful evaluation of individual risk-benefit is necessary for patients with significant residual thyroid tissue. Enlargement of residual thyroid tissue or metastases can occur following treatment with Thyrogen®. This may lead to acute symptoms, which depend on the anatomical location of the issue. For example, hemoptysis, hemiparesis or loss of vision have occurred in patients with CNS metastases. Levothyroxine, respiratory distress requiring tracheotomy, and pain at the site of metastases have also been reported after Thyrogen® administration. It is recommended that pre-treatment with corticosteroids be considered for patients in whom local tumour expansion may compromise vital anatomical structures. Pregnancy and lactation: Effects of Thyrogen on the reproductive organs and on the foetus are unknown. Thyrogen® is contra-indicated in pregnancy when used in procedures that involve exposure of the foetus to radioactive material. Patients should not breast feed. Contamination: Hypersensitivity to bovine or human thyroid stimulating hormone or to any of the excipients. Pregnancy, Legal classification: POM (prescription only medicine). List Price: £533.94 per pack of 2 vials. Marketing Authorisation Holder: Genzyme Europe B.V., Gooroom 10, 1411 DD Naarden, The Netherlands, EU/097/222/001, EU/99/122/002. Further information is available from Genzyme Ltd, 4620 Kinggate, Cascade Way, Oxford Business Park South, Oxford OX4 2SU. Date of Preparation: February 2011. (Status of SmPC: January 2018).

Adverse events should be reported. Reporting forms and information can be found at www.yellowcard.gov.uk. Adverse events should also be reported to Genzyme at tel: +44 (0)1865 405200.
The abstracts submitted were marked by the Abstract Marking panel selected by the Programme Committee.

**Programme Committee**

M Korbonits  
K Chapman  
S Orme  
M Druce  
C Duncan  
A Munir  
A Toogood  
J Wass

**Members**

A Allahabadia  
W Arlt  
P Clayton  
E Davies  
W Dhillo  
M Druce  
K Meeran  
A Munir  
A Toogood  
J Wass

**Abstract Marking Panel**

J Ahlquist Southend  
F Ahmed Glasgow  
B Allolio Wuerzburg, Germany  
R Amin London  
B Andrew Edinburgh  
J Aylwin London  
K Bailey Liverpool  
A Bates Birmingham  
K Boelort Birmingham  
W Bouloux London  
N Bridges London  
J Burrin London  
K Chapman Edinburgh  
T Cheetham Newcastle upon Tyne  
P Clark Birmingham  
P Clayton Manchester  
J Compston Cambridge  
M Cooper Birmingham  
S Cox Torquay  
M Dattani London  
E Davies Glasgow  
J Davis Manchester  
A Lam Toronto, Canada  
G Lavery Birmingham  
G Leese Dundee  
A Levy Bristol  
M Levy Leicester  
N Martin London  
C Mcardle Bristol  
C Mcnulty Edinburgh  
J Miell London  
D Morganstein London  
D Morris Ipswich  
J Morris Oxford  
N Morton Edinburgh  
J Monday Portsmouth  
K Murphy London  
R Murray Leeds  
J Newell-Price Sheffield  
S Orme Leeds  
S Peacey Bradford  
S Pearce Newcastle  
P Pickett Shrewsbury  
R Quinton Newcastle upon Tyne  
S Razvi Gateshead  
A Rees Cardiff  
E Scott Leeds  
P Selby Manchester  
D Shapiro Glasgow  
L Shepherd Birmingham  
P Squires Warwick  
C Stewart Cheshire  
N Taylor London  
M Thomas London  
J Tomlinson Birmingham  
B Vaidya Plymouth  
M Vanderpump London  
B Walker Edinburgh  
E Ward Leeds  
J Wass Oxford  
M Westwood Manchester  
A Wheatman Sheffield  
G Williams London
CORPORATE SUPPORT
The Society for Endocrinology would like to thank its Corporate Supporters, whose commitment is greatly appreciated.

Platinum Supporters:
BioScientifica Ltd
Ipsen Ltd
Pfizer Ltd

Gold Supporters:
Bayer Schering Pharma
Ferring Pharmaceuticals Ltd
Merck Serono
Novartis Pharmaceuticals UK Ltd
Novo Nordisk Ltd

Silver Supporters:
Almirall Ltd
Amgen Ltd
Eli Lilly and Company Ltd
Sandoz Ltd

The ‘Meet the Expert’ sessions and the ‘Clinical Management Workshops’ are generously supported by Clinical Endocrinology.
CONTENTS

Society for Endocrinology BES 2011

PLENARY LECTURERS' BIOGRAPHICAL NOTES

PLENARY LECTURES

Society for Endocrinology Dale Medal Lecture ........................................ PL1
Society for Endocrinology Hoffenberg International Medal Lecture .............. PL2
Society for Endocrinology Transatlantic Medal Lecture .......................... PL3
Society for Endocrinology European Medal Lecture 2010 ......................... PL4
Society for Endocrinology European Medal Lecture 2011 ........................ PL5
Society for Endocrinology Medal Lecture ................................................ PL6
The British Thyroid Association Pitt Rivers Lecture .................................. PL7
Clinical Endocrinology Trust Visiting Professor Lecture ........................ PL8
Clinical Endocrinology Trust Lecture .................................................... PL9

SYMPOSIA

All you need to know about the genetics of diabetes (types 1 and 2 and MODY) ................................................. S1.1–S1.4
Novel pathways and treatments in neuroendocrine tumours ....................... S2.1–S2.4
Fat endocrinology: disorders of adipose tissue and lipids important to the endocrinologist ....................................... S3.1–S3.4
Endocrine regulation of ageing ................................................................. S4.1–S4.4
The novel role of primary cilia in endocrine disease and obesity .................. S5.1–S5.4
Novel application of thyroid hormone analogues: thyroid hormones, thinking outside the capsule .......................... S6.1–S6.4
Eat, bond, reproduce – what the hypothalamus dictates ............................ S7.1–S7.4
Hormones and bone metabolism ............................................................ S8.1–S8.4
Doping: performance enhancing substances in sport and their detection ....... S9.1–S9.4

CLINICAL MANAGEMENT WORKSHOPS

The management of difficult Graves’ disease ........................................... CM1.1–CM1.4
Endocrine problems in pregnancy ......................................................... CM2.1–CM2.4
Pituitary radiotherapy: what are the options? ........................................ CM3.1–CM3.4
Management of disorders of sex development (DSD) across the lifespan ... CM4.1–CM4.4

APPLIED PHYSIOLOGY WORKSHOP

Seeing is believing – cutting edge in vivo cell imaging .............................. AP1.1–AP1.3

CLINICAL GUIDELINES ........................................................................ CG1.1

SPECIAL INTEREST GROUPS

Bone and mineral special interest group ................................................. SIG1.1–SIG1.4
Obesity special interest group ............................................................... SIG2.1–SIG2.4
Laboratory aspects of clinical endocrinology special interest group ............ SIG3.1–SIG3.4

MEET THE EXPERT SESSIONS .......................................................... MTE1–MTE9
NURSE SESSION
Turner/Klinefelter’s/Noonan syndrome: case presentations N1.1–N1.4
Hyperthyroidism: case presentations N2.1–N2.4

YOUNG ENDOCRINOLOGISTS SESSION
Young endocrinologists’ prize lectures YEP1.1–YEP1.2
A successful research career YE1.1–YE1.4

SENIOR ENDOCRINOLOGISTS SESSION SE1.1–SE1.6

ORAL COMMUNICATIONS
Young Endocrinologists prize session OC1.1–OC1.8
Steroids OC2.1–OC2.8
Pituitary and thyroid OC3.1–OC3.8
Bone and diabetes OC4.1–OC4.8
Reproduction and fetal programming OC5.1–OC5.8

POSTER PRESENTATIONS
Bone P1–P26
Clinical biochemistry P27–P108
Cytokines, growth factors, neuroendocrinology and behaviour P109–P119
Diabetes, metabolism and cardiovascular P120–P167
Endocrine tumours and neoplasia P168–P203
Growth and development P204–P208
Nursing practise P209–P221
Pituitary P222–P262
Reproduction P263–P282
Steroids P283–P311
Thyroid P312–P354

INDEX OF AUTHORS
Plenary Lecturers’ Biographical Notes
Society for Endocrinology Dale Medal Lecture

E R Simpson, Prince Henry’s Institute, Clayton, Victoria, Australia

Dr Evan Simpson is a native of Edinburgh, Scotland. He has had a long interest in the basic biology of estrogen biosynthesis, especially its relationship to breast cancer.

His group was the first to clone and characterize the aromatase gene and to show the unique use of tissue-specific promoters to regulate tissue-specific expression of the gene. This led in turn to the concept of breast-specific inhibitors of aromatase expression as a novel therapeutic modality for breast cancer in post-menopausal women.

His group’s development of the aromatase knockout mouse, as a model of estrogen deficiency, provided insights into the role of estrogens in the physiology and pathophysiology of both male and females.

More recently his group is working to understand the molecular mechanisms underlying the relationship between obesity, aging and breast cancer.
Society for Endocrinology Hoffenberg International Medal Lecture

Peter J Fuller, Prince Henry’s Institute, Clayton, Victoria, Australia

Professor Fuller is an NHMRC Senior Principal Research Fellow at Prince Henry’s Institute of Medical Research in Melbourne where is the Associate Director and Head of the Steroid Receptor Biology Group. He is also Director of the Endocrinology Unit at the Monash Medical Centre/Southern Health and an Adjunct Professor in Medicine and Biochemistry & Molecular Biology at Monash University.

He trained in Melbourne in both clinical endocrinology and molecular endocrinology before postdoctoral training at the Massachusetts General Hospital in Boston.

He was awarded a Wellcome Trust Australian Senior Research Fellowship in 1987 and has received the Eric Susman Prize from the Royal Australasian College of Physicians.

He has served on the Council and as President of the Endocrine Society of Australia; in 2008 he was made a Life Member of the Society. He has served as a Receiving Editor for Clinical Endocrinology and is currently an Editor of Endocrinology.

He has published over 170 original articles, reviews and book chapters.

His research interests lie primarily in understanding the molecular mechanisms of adrenal steroid hormone action. His laboratory also has collaborative programmes to study the molecular pathogenesis of granulosa cell tumours of the ovary.
Society for Endocrinology Transatlantic Medal Lecture

J J Kopchick, Ohio University, Athens, Ohio, USA

Dr John J Kopchick is an internationally recognized leader in the growth hormone (GH) field. Since 1987, he has held the Milton and Lawrence H Goll Eminent Scholar Professorship in Molecular and Cellular Biology and directs the Growth/Obesity/Diabetes Section of the Edison Biotechnology Institute at Ohio University in Athens, Ohio. He also is Professor in the Biomedical Sciences Department in the College of Osteopathic Medicine at Ohio University.

In 1989, Dr. Kopchick and his group were the first to discover and characterize the molecular aspects of GH receptor antagonists, an accomplishment for which he and Ohio University were awarded several U.S. and European patents. He was instrumental in founding a company, Sensus, which applied his laboratory discovery to development of a drug that has been evaluated in clinical trials for acromegaly. Dr Kopchick has been involved in the start up of two additional biotechnology companies. The latest, DiAtheugen, focuses on the discovery of diagnostics, therapeutics, and therapeutic targets in the obesity and diabetes field.

Born in Punxsutawney, Pennsylvania, Dr Kopchick spent most of his early years in Indiana, Pennsylvania. He received both his bachelor’s degree in biology and master’s degree in biology and chemistry from Indiana University of Pennsylvania. In 1975, he enrolled in the Graduate School of Biomedical Sciences, University of Texas System Cancer Center in Houston, Texas and began research under the supervision of Dr Arlinghaus. While a graduate student at M.D. Anderson, he was awarded a Rosalie B Hite scholarship that supported his studies. His dissertation described the biosynthesis of Rauscher murine leukemia virus reverse transcriptase. He was awarded his Ph.D. in 1980.

After completing his PhD, Dr Kopchick continued research training as a postdoctoral fellow at the Roche Institute of Molecular Biology in Nutley, New Jersey where he received a National Cancer Institute Postdoctoral Fellowship award to support his work. He then spent almost five years at Merck Sharp & Dohme Research Laboratories. At Merck, he progressed to the level of Director and developed a system built around cloning and expression of GH genes, which he continues to study as director of the Edison Biotechnology Institute.

Dr. Kopchick has published more than 290 scientific articles and 310 published abstracts in the area of growth, obesity and diabetes. His publication impact factor (h rating) is 44. Twelve patents have been granted based on his work with several more pending. He has also advised several undergraduate, post graduate and doctoral students. He has served on the Editorial Boards of several journals including The Journal of Biological Chemistry, Endocrinology, Molecular Endocrinology, and currently serves on the editorial board of GH & IGF-1 Research, and The Journal of Biological Chemistry, and The Polish Journal of Endocrinology. He is also President Elect of the Growth Hormone Research Society.

Dr. Kopchick has received many awards including The Outstanding Alumnus award from Indiana University of Pennsylvania, Indiana, PA (2002); The Outstanding Alumnus award from M.D. Anderson Hospital and Tumor Institute, Houston, TX (2002); The Outstanding Alumnus Award from the Graduate School of Biomedical Sciences, University of Texas, Houston (2006), The New York College of Osteopathic Medicine’s Riland Award (2007); an Honorary Dr of Sciences Degree from Indiana University of Pennsylvania, Indiana, PA (2008), and the Phillips Medal of Honor Award from the Ohio University of Osteopathic Medicine (2010).
Society for Endocrinology European Medal Lecture 2010

Bruno Allolio, University of Würzburg, Germany

Bruno Allolio is Professor of Medicine at the University of Würzburg and Head of the Department of Endocrinology at the University Hospital Würzburg.

He trained in Cologne in both clinical and experimental endocrinology. For postdoctoral studies he worked at Bartholomew’s Hospital, London, and at the National Institutes of Health, Bethesda. After Habilitation in 1988 he moved to Würzburg in 1992.

In 1988 he received the Schöller-Junkmann Award of the German Society of Endocrinology. He has served as Editor for the European Journal of Endocrinology and the Journal of Clinical Endocrinology and Metabolism. He was the first media spokesman for the German Society of Endocrinology and he served in the first executive committee of the European Society of Endocrinology.

He has published over 300 original articles, reviews and book chapters.

His research interests focus on adrenal disorders, mineral metabolism, and recently also on hyponatraemia. He is a founding member of the European Network for the Study of Adrenal Tumours (ENSAT) and serves on the Expert Commission of the Deutsche Forschungsgemeinschaft.
Society for Endocrinology European Medal Lecture 2011

Xavier Bertagna, Cochin Hospital, Paris, France

Xavier Bertagna is Professor of Endocrinology, Chief of the Department of Endocrinology of Cochin hospital, Faculté de Médecine René Descartes, Université Paris, Paris, France.

He trained at the Clinical Research Center (Dr. M. Chrétien), Montreal, Canada and at the Endocrine Division, Vanderbilt Medical School (Dr. D. Orth), Nashville, TN, USA.

His research interest is in endocrine tumors, adrenal cancer, Cushing’s syndrome, disorders of the pituitary-adrenal axis, polypeptide hormone precursors, and takes place in the Endocrinology Department of Institut Cochin (INSERM U-567), France.

He was at the origin of two important Clinical and Scientific Networks devoted to the study of adrenal tumors, namely, the COMETE network in France and the ENS@T Network in Europe.

Xavier Bertagna is involved in many National and International professional bodies. He has been a member of the Executive Committee of the International Society of Endocrinology for two consecutive terms.
Society for Endocrinology Medal Lecture

G R Williams, Imperial College London, London, UK

Graham R Williams obtained a BSc in Anatomy and MBBS from St Thomas’s Hospital, London and undertook PhD studies in Molecular Endocrinology at Birmingham University. He trained as a Howard Hughes and MRC Fellow at Harvard Medical School, USA and was an MRC Clinician Scientist Fellow in Birmingham. He was appointed Senior Lecturer at the Royal Postgraduate Medical School and received an MRC Career Establishment Award. He is currently Professor of Endocrinology at Imperial College London and Honorary Consultant Physician at Hammersmith Hospital.

Professor Williams is Treasurer and a Council Member of the Society for Endocrinology, a current member of the European Thyroid Association Executive and former Member of the British Thyroid Association Committee. He is currently on the Editorial Boards of Endocrinology, Thyroid and Journal of Neuroendocrinology and sits on the EORTC Endocrine Task Force for Thyroid Cancer, the World Thyroid Foundation Advisory Committee and the Wellcome Trust Physiological Sciences Funding Committee.

His research is focused on the molecular mechanisms of thyroid hormone action in bone and cartilage. A major aim is to train and support young endocrinologists for the future, and fellows and post-docs in the Molecular Endocrinology Laboratory have received 24 prizes for their research.
British Thyroid Association Pitt-Rivers Lecture

Samuel Refetoff, University of Chicago, IL, USA

Samuel Refetoff is known for his discovery (1967) of resistance to thyroid hormone (Refetoff Syndrome) and elucidation of its genetic and molecular basis (1989/92).

Devoted to the study of inherited thyroid disorders, his laboratory was first to identify mutations in: serum TH transport proteins [TBG (1989) and albumin (1994)]; the TSH receptor producing resistance to TSH (1995); and two syndromic thyroid defects combining neuropsychological and thyroid abnormalities caused by mutations in the TTF1 (2002) and the MCT8 (2004) genes.

In 2005 his laboratory identified a defect of TH metabolism caused by mutations in the SBP2 gene, which is involved in the synthesis of selenoproteins.

A graduate of McGill University, Dr. Refetoff is professor of medicine, pediatrics and genetics at the University of Chicago.

He is author of over 470 publications and recipient of numerous national and international prizes, two NIH MERIT awards (1989/2006) and two honorary doctorate degrees.
James A. Fagin is Chief of the Endocrine Service and a Member of the Human Oncology and Pathogenesis Program at Memorial Sloan-Kettering Cancer Center in New York, and a Professor of Medicine at Weill Medical College of Cornell University. He was formerly the James Heady Professor of Medicine and Director of the Division of Endocrinology and Metabolism at the University of Cincinnati. He has had a long standing interest in the pathogenesis of thyroid neoplasms, and his laboratory focuses on thyroid cancer genetics, on the development of mouse models to understand the biology of these tumors, and on the identification of specific therapies directed at key oncoproteins that drive the disease.

He was a member and then the chair of the NIH Endocrinology Study Section (1998–2002). In addition to serving on a number of Editorial Boards, he is a former Associate Editor of Endocrinology, former Editor (Americas) for Clinical Endocrinology (Oxford), and recently retired as Editor-in-Chief of Endocrine-Related Cancer. Dr. Fagin is a member of the American Society of Clinical Investigation and the Association of American Physicians. His honors include the Merck Prize of the European Thyroid Association, and the Sidney H. Ingbar Distinguished Lectureship Award of the American Thyroid Association. He is also the President-elect of the American Thyroid Association.
Clinical Endocrinology Trust Lecture

John S Bevan, Department of Endocrinology, Aberdeen Royal Infirmary, Foresterhill, Aberdeen AB25 2ZN, UK

John S Bevan is senior Consultant Endocrinologist at Aberdeen Royal Infirmary and Honorary Professor of Endocrinology at Aberdeen University. He qualified MB ChB, with Honours, from Edinburgh University in 1978 and his clinical training in Medicine & Endocrinology took place in Edinburgh, Oxford and Cardiff, 1979–91. Whilst in Oxford he was awarded an MRC Training Fellowship and wrote his MD thesis on dopamine agonist treatment of large pituitary tumours. As Head of Endocrinology at Aberdeen Royal Infirmary, Dr Bevan has responsibility for coordinating endocrine care across the North of Scotland, including the Orkney and Shetland Islands. He has been a Council Member of the Royal College of Physicians of Edinburgh since 2006 and Chair of the Scottish Paediatric Endocrinology Managed Clinical Network since 2009.

Professor Bevan has been a member of the Clinical Committee of the Society for Endocrinology since it was first convened in 1997. During 2002–09 he was National Coordinator for Peer Review of UK Clinical Endocrinology. He has twice been a member of the UK Acromegaly Database Steering Group. He was Secretary/Treasurer to the Clinical Endocrinology Trust, 2006–10. He has been a Senior Editor of Clinical Endocrinology since 2008, having previously been Associate Editor, 1994–2004.

Professor Bevan has a longstanding interest in clinical neuroendocrinology, particularly the use of medical therapies for pituitary tumours; he has written and lectured extensively on prolactinoma, non-functioning pituitary tumour and acromegaly.
Plenary Lecturers
Society for Endocrinology Dale Medal Lecture

PL1

Fat, sex hormones and breast cancer
Evan Simpson & Kristy Brown
Department of Biochemistry and Physiology, Prince Henry’s Institute, Monash University, Clayton, Victoria 3168, Australia.

After the menopausal transition, the ovaries cease to make oestrogens, yet the incidence of breast cancer increases with aging and the majority of these tumours are ER positive. So, where is the oestrogen driving this tumour development coming from? Several extra-gonadal sites synthesise oestrogens from circulating C19 steroids, such as bone, brain, and adipose. The largest of these deposits is the adipose tissue, and increased BMI is associated with increased breast cancer risk, so, given the global ‘pandemic’ of obesity, we are faced with the daunting prospect that tens of millions more women may be at risk of breast cancer in their later years than was previously thought. Yet the mechanisms linking obesity to cancer risk are not completely understood. Factors increased in obesity such as leptin and insulin appear to increase breast cancer risk, probably via activation of the Akt/mTOR/HIF1α/SREBP pathways, whereas adiponectin has been shown to decrease the risk in a number of studies. This appears to be due, in part, to activation of the LKB1/AMPK pathway. The anti-diabetic drug metformin has also been shown to decrease the risk of breast cancer, and it also acts to stimulate AMPK. Given the capacity of adipose tissue to express aromatase, the enzyme responsible for oestrogen biosynthesis, we looked for a link between obesity and increased aromatase expression in breast adipose. In the presence of a tumour, aromatase expression locally in the breast is stimulated by inflammatory mediators produced by the tumour such as PGE_2 and TNFα. We observed that AMPK is a potent inhibitor of aromatase expression stimulated by PGE_2 in breast adipose-stromal cells. The mechanism involves sequestration in the cytoplasm by phosphorylation of a CREB coactivator, CRTC2. The LKB1/AMPK pathway is inhibited in breast cancer cells, and CRTC2 as well as CRTC4, both are increased in obesity, this provides a new mechanism whereby obesity increases breast cancer risk. Moreover, we observed that metformin also inhibits aromatase expression in breast stromal cells, associated with an increase in LKB1/AMPK activity. A number of trials are currently underway to establish if metformin could find utility as a breast cancer therapeutic agent, and we have commenced a study to establish if metformin treatment in the neoadjuvant and adjuvant settings results in an increase in the activity of the LKB1/AMPK pathway and a decrease in the expression of aromatase in the female breast. An advantage of metformin as an inhibitor of aromatase expression is that such inhibition is breast-specific in the post-menopausal woman. This is due to the unique use of tissue-specific promoters by the aromatase gene to regulate its tissue-specific expression. Currently phase III aromatase inhibitors are used as hormonal therapy for breast cancer, but their use has significant side-effects including bone loss hot flushes and arthralgia, due to global inhibition of aromatase catalytic activity. Use of AMPK activators such as metformin should inhibit aromatase expression uniquely in the breast, but leave protected other sites of expression such as bone and brain, where oestrogens have important functions.

Society for Endocrinology Hoffenberg International Medal Lecture

PL2

Aldosterone action and the mineralocorticoid receptor
Peter Fuller1,2
1Prince Henry’s Institute of Medical Research, Clayton, Victoria, Australia; 2Department of Endocrinology, Southern Health, Clayton, Victoria, Australia; 3Monash University, Clayton, Victoria, Australia.

The mineralocorticoid receptor (MR) differs from the other steroid receptors in that it responds to two physiological ligands, aldosterone and cortisol. In epithelial tissues, aldosterone selectivity is determined by the activity of 11β-hydroxysteroid dehydrogenase type II. The aldosterone-induced genes that mediate sodium flux in epithelial tissues are now well characterised. In other tissues, including the heart and regions of the CNS, cortisol is the primary ligand for the MR, in some tissues cortisol may act as an antagonist. Clinical trials have demonstrated the potential of MR antagonists in the treatment of cardiovascular disease however their use has been limited by concurrent hyperkalaemia. The mechanisms of these therapeutic effects are currently being addressed by the use of animal models including tissue-specific deletions of the MR. In order to better target the MR an understanding of the structural determinants of tissue and ligand-specific MR activation is being sought. We have focused on interactions of the ligand-binding domain (LBD) with ligand, with the N-terminal domain and with putative co-regulatory molecules. Both agonist and antagonist binding has been characterised using chimera between the human (h)MR LBD and both the glucocorticoid receptor and the synthetic (s)MR together with a similar chimera between the N-terminus and C-terminus/LBD (N/C-interaction) observed in the MR is aldosterone-dependent but is unexpectedly antagonised by cortisol and DOC in the hMR but not the sMR. Nuclear receptor mediated transactivation is critically dependent on, and modulated by the co-regulatory molecules. Yeast-2 hybrid screens with the MR LBD have identified proteins which interact in the presence of either aldosterone or cortisol but not both. These have been confirmed as coactivators of the full-length hMR in a transactivation assay. The successful identification of ligand-specific interactions of the MR may provide the basis for the development of novel MR ligands with tissue specificity.

Society for Endocrinology Transatlantic Medal Lecture

PL3

GH, GR receptor antagonists, GH receptor ‘knock-outs’: a story of fat old mice
John Kopchick
Ohio University, Athens, Ohio, USA.

In this talk I will describe several genes that have been implicated in the action of GH as it relates to aging. Much of the data is derived from two dwarf and one giant strain of mice produced in our laboratory that possess very different life spans. One of the dwarf lines contains a disruption of the GH receptor and binding protein gene (GHR/βP) (PNAS, 94:13215–13220, 1997). Homozygous GHR/βP ‘knockout’ mice (GHRβP–/–) show severe postnatal growth retardation, absence of the GHR and GHRBP mRNA and protein, low levels of serum IGF1 and IGFBP3 and elevated levels of GH. These parameters are characteristic of the GH insensitivity phenotype typical of humans with Laron syndrome. Surprisingly, the life span of the GHRβP–/– mice is significantly longer than +/- or +/- littermates. These mice also possess near normal levels of serum glucose with very low levels of insulin and are extremely sensitive to insulin’s action. Furthermore, the mice are resistant to diabetes-induced glomerulosclerosis. We also have discovered a GH receptor antagonist (A). A brief history of this discovery along with clinical implications will be presented. Expression of the GHA transgene results in another dwarf strain of mice. These mice have low levels of IGF1 but do not possess an increased life expectancy. Additionally, we have produced giant GH transgenic mice that die prematurely of liver, heart and kidney pathology. A comparison of the endocrine parameters of these three mouse lines will be shown. Also, these three strains of mice were found to have very different adipose depot disposition profiles. The interaction of adipose tissue in the aging process will be discussed. Finally, using both genomic and proteomic approaches, we have identified several genes/proteins whose levels change as a function of 1) mouse age, 2) diet induced type 2 diabetes and 3) GH treatment of GH deficient patients. This work was supported by the State of Ohio’s Eminent Scholar Program which includes a grant from Milton and Lawrence Gollf; WADA; and NIH grants, R15DK075436, RO1 AG19899-05, and P01 AG031736-01A1.

Society for Endocrinology European Medal Lecture 2010

PL4

Adrenocortical carcinoma: advance through international cooperation
Bruno Allolio
Department of Endocrinology, University of Wuerzburg, 97080 Wuerzburg, Germany.

Adrenocortical carcinoma (ACC) is a heterogeneous neoplasm with an incompletely understood pathogenesis and an unsatisfactory prognosis. However, international initiatives like the European Network for the Study of Adrenal Tumours (ENSAT) and international trial consortia recently led to improved diagnosis and treatment. Activated signalling pathways primarily involved in adrenal development like IGF2, SFI, and β-catenin pathways play also a key role in the ACC development and provide important diagnostic and prognostic information. A standardized comprehensive diagnostic work up is now recommended by ENSAT (www.ensat.org/ACC.htm). A major diagnostic advance is 18F-FDG-PET, as virtually all ACC patients demonstrate high uptake leading to high diagnostic sensitivity. In addition, 13C-metomidate or 123I-iodo-tyrosine scintigraphy using the radiotracer CYP11B enzymatic conferring high specificity to ACC imaging. Survival in ACC is highly stage dependent with the new ENSAT staging system providing superior prognostic information compared
to the WHO/UICC system. Surgery aiming at R0 resection remains the treatment of choice and requires an experienced surgeon. Smaller ACCs (diameter <9 cm) may be removed safely by minimal invasive surgery. To establish the individual risk, adjuvant therapy of the tumor bed may be added. Cytotoxic drugs (such as platinum compounds) will be added to mitotane in advanced disease. Results of the international FIRM-ACt trial, the largest ever trial in patients with advanced ACC, will become available in early 2011 and will establish a benchmark therapy. New targeted therapies (e.g. IGF1 receptor antagonists, [123I-iodomethoxylate]) are under investigation and may soon lead to improved treatment options. Furthermore, the recently funded (FP7 program) ENSAT-CANCER consortium holds great promise to keep the recently gained momentum in ACC research.

The British Thyroid Association Pitt-Rivers Lecture
PL7
An expanded view on resistance to thyroid hormone
Samuel Refetoff
The University of Chicago, Chicago, Illinois, USA.

At least six major steps are required for secreted thyroid hormone to exert its action on target tissues: thyroid hormone transport into cells, intracellular activation of the prohormone tyrosine, transfer of the hormone from the cytoplasm to the nucleus, intact thyroid hormone receptors and intact nuclear and cytosolic machinery required for the mediation of thyroid hormone action. Mutations interfering with three of these steps have been so far identified and are the subject of this presentation. The first recognized defect, causing resistance to thyroid hormone (RTH), involves the thyroid hormone receptor β (TRβ) gene and carries the acronym, RTH. Occurring in ~1 of 40 000 newborn, affected subjects belonging to more than 400 families surpass 1500. The gene defect remains unknown in 15% of subjects with RTH. Two novel syndromes causing reduced sensitivity to thyroid hormone were identified in the last 5 years. One, producing severe psychomotor defects in more than 150 males from 57 families, is caused by mutations in the monocarboxylate transporter 8 (MCT8 or SLC16A2), a thyroid hormone cell-membrane transporter of particular importance in early brain development. The second defect, affecting the intracellular metabolism of thyroid hormone is caused by mutations in the selenocysteine insertion sequence binding protein 2 (SECISBP2 or SBP2) gene required for the synthesis of selenoproteins, including the iodothyronine deiodinases. Eight such individuals, belonging to unrelated families, have been identified. They manifest, in addition to thyroid test abnormalities, defects of variable magnitude in the reproductive, muscular and immune systems. Most challenging is the approach to therapy, when simple hormonal substitution is ineffective.

Partly supported by the Clinical Endocrinology Trust.

Society for Endocrinology Trust Visiting Professor Lecture
PL8
Genetics of thyroid cancer: clinical and therapeutic implications
James Fuglin
Memorial Sloan Kettering Cancer Center, New York, USA.

Our understanding of the genetic abnormalities associated with thyroid cancers has grown significantly over the past decade. Thus, papillary thyroid cancers (PTC) have non-overlapping activating mutations genes encoding the growth factor receptors RET or NTRK, the three isoforms of RAS, or of BRAF, which altogether are found in ~70% of cases. In addition, mutations of effectors in the phosphoinositol-3-kinase (PI3K) pathway are present at various stages of the disease, but in particular in advanced thyroid cancers. There is also a strong genotypetype-phenotype relationship in thyroid cancers, with specific mutations associated with particular histological variants, and more importantly, with the biological behaviour and prognosis of the cancers. The BRAF mutation, in particular, is associated with invasive PTC, which more often become radioactive iodine refractory. The relevance of many of the genotype–phenotype correlations first identified through association studies in human specimens is now supported by mouse models, in which oncogene activation through transgenic or gene targeting approaches recapitulates the main features of various forms of the disease. It is now legitimate to ask whether this information can be leveraged to
make clinical decisions, and improve the care of patients with the disease. For example, FNA of thyroid nodules discriminates between benign and malignant thyroid cancers in most instances. Several groups have shown that immuno-histochemical or genetic assays can refine the accuracy of cytopathological diagnoses. Most oncogenic mutations in thyroid cancer result in constitutively active kinases, which provide tractable targets for pharmacological inhibition. This is based on the assumption that cancers will remain dependent on the activity of the initiating oncoprotein for viability, despite accumulating further genetic damage during the course of their evolution. As a proof-of-principle, the multi-kinase inhibitor AZD6474, which inhibits RET, EGFR and KDR, showed promising evidence of activity in a phase 3 clinical trial of patients with medullary thyroid cancer. However, it is not clear that the beneficial effects of this agent are due to its inhibition of RET kinase, or to effects on other kinases. Several clinical trials incorporating patients with papillary, follicular and Hurthle cell carcinoma with multi kinase inhibitors have also been reported. Two studies with sorafenib, an inhibitor of KDR, RET and possibly RAF, showed a high percentage of patients with disease stabilization and some partial responses, particularly in PTC. Although sorafenib was developed as a RAF inhibitor, it is not clear if it is sufficiently potent to block the activity of mutant BRAF \( \text{in vivo} \). Indeed, it has been proposed that the activity of sorafenib, as well as that of other multi kinase inhibitors such as axitinib and motesanib, may be due to their antiangiogenic activity, since they are all inhibitors of KDR, the receptor for vascular endothelial growth factor. Despite the promising results of these early trials, many important questions remain. It is not clear if the favorable responses will prove to be durable. Moreover, no study so far has been designed to examine impact on mortality. In view of these uncertainties, these drugs should be considered only for patients with rapidly progressing metastatic disease, ideally as part of a clinical trial. Although these compounds are quite well tolerated, they do have significant side effects, and many participants in the trials required dose reductions, drug holidays, and in some cases were unable to continue on the study. In other tumor types, the type of genetic mutation harboured by the cancer predicts response to therapy with specific kinase inhibitors. Despite this, most trials of thyroid cancers derived from follicular cells were not designed to examine whether specific oncogenic mutations might determine treatment outcome, a shortcoming that will hopefully be corrected as this promising research field continues to develop.

---

**Clinical Endocrinology Trust Lecture**

**PL9**

**Pituitary tumours: the goal is shrinking!**

John S Bevan

Aberdeen Royal Infirmary, Aberdeen, UK.

In 2011 is the 40th anniversary of prolactin (PRL) characterisation as a distinct hormone. Only 30 years ago most patients with large pituitary tumours received primary surgery (often transcranial) followed by routine radiotherapy – treatments associated with significant morbidity and hypopituitarism. Much therapeutic progress has been made; effective medical treatments now exist for many pituitary tumour subtypes, particularly the use of long-acting dopamine agonists (DA) and somatostatin analogues (SA), with potential for growth control and tumour shrinkage (TS). Typical DA responses of a macroadenoma include rapid PRL reduction, TS (within days/weeks), visual improvement (often within hours/days) and recovery of normal pituitary function. Up to 90% of patients show these responses and most tumours shrink by at least 50%. Lactotroph cells shrink by over 50% with notable reductions in the cytoplasmic and RER components. In acromegaly, SAs control tumour growth in the majority and induce significant TS in \( \geq 50\% \) of de novo patients, with an average tumour volume reduction of \( \geq 50\% \), but there is a wide range of TS in unselected GH-tumours. Somatotroph cell size reduction is modest, but diminished proliferation and angiogenesis have been demonstrated. SA are also effective in de novo patients with TSH-secreting adenomas with significant TS in the majority. Many non-functioning pituitary tumours express D2 dopamine receptors (albeit fewer than in prolactinomas) and DA therapy restrains tumour growth in up to 70%, although only a minority show marked shrinkage. Increasingly, dopamine and somatostatin receptor subtype analysis will guide specific medical therapies for individual tumours, not only with the agents currently available, but also with multitargeted SA (such as pasireotide) and chimeric molecules (such as dopastatin). Despite their frequent clinical effectiveness, issues of drug safety, value for money and long-term cure rates associated with medical treatments for pituitary tumours continue to be evaluated and debated.

---

Generously supported by the Clinical Endocrinology Trust.
Symposia
**S1.1**

**Immune mechanisms involved in type 1 diabetes: insights from genetics**

V Plagnol
University College London Genetics Institute, London, UK.

Recent advances in genotyping technologies combined with large scale recruitment of case-control cohorts have enabled the development of the genome-wide association (GWA) study design. As a consequence of this onslaught, work, 64 single nucleotide polymorphisms (SNPs) located in 53 chromosome regions have now been associated with type 1 diabetes (T1D) risk (see www.t1dbase.org). These findings provide an unbiased assessment of the genetic architecture of T1D and I will give an overview of these data. In addition, the comparison of these results with similar findings obtained from additional GWA scans designed for other auto-immune disease, as well as for autoantibody data in T1D patients, provides further insights into the shared aetiology of autoimmune pathologies. Arguably one of the most striking conclusions is the indication that viral infections are involved in the pathogenesis of T1D as well as other autoimmune disorders.

**S1.2**

**The diverse phenotypes of K<sub>A TP</sub> channel mutations**

Sian Ellard
Peninsula Medical School, University of Exeter, Exeter, UK.

In recent years, there has been significant progress in defining the genetic aetiology of neonatal diabetes (NDM). It is likely that all cases result from single gene disorders since markers of autoimmunity associated with polygenic type 1 diabetes are rare in patients diagnosed before 6 months.

Activating mutations in the KCNJ11 and ABCC8 genes encoding the Kir6.2 and SUR1 subunits of the β-cell ATP sensitive potassium (K<sub>A TP</sub>) channel are the most common cause of neonatal diabetes, accounting for around 40% of cases. The majority (~90%) of patients can achieve improved glycaemic control on high dose sulphonylureas.

The mutation severity defines the phenotype which ranges from isolated transient neonatal diabetes to DEND syndrome (severe developmental delay, epilepsy and permanent neonatal diabetes). Around 20% of patients with activating K<sub>A TP</sub> channel mutations have mild to moderate developmental delay and case reports have described improved neurological function in some of these patients following transfer to sulphonylureas.

Some patients with transient neonatal diabetes caused by a K<sub>A TP</sub> channel mutation have inherited the mutation from a parent who did not present with diabetes in the neonatal period, but developed permanent diabetes in later life. Analysis of a cohort of patients referred for HNF1A/4A MODY testing identified activating ABCC8 mutations in 6/86 cases (7%). The identification of specific subtypes of monogenic diabetes not only provides accurate information regarding inheritance and prognosis, but can inform treatment decisions and improve clinical outcome.

**S1.3**

**How to find all the cases of MODY in your diabetes clinic…**

Katharine Owen
University of Oxford, Oxford, UK.

Maturity-onset diabetes of the young (MODY) is a group of monogenic disorders of β-cell function which causes diabetes in young adults. Diagnosing MODY is important as the different subtypes have distinct first line treatments, e.g. low dose sulphonylureas in HNF1A mutations while no treatment is required in GCK mutations. This can lead to treatment changes including discontinuing insulin therapy. First degree family members can also be offered diabetes screening and genetic testing. However, there are considerable challenges in differentiating MODY from type 1 and type 2 diabetes and this means that an estimated 80-90% of cases in the UK are undiagnosed and on average there is a delay of around 15 years after diagnosis of diabetes before a genetic diagnosis is established.

Finding economic, widely-available non-genetic biomarkers that can be used for screening to find those at high risk of MODY would be greatly advantageous. This lecture describes the recent progress that has been made in this area.

One approach to finding biomarkers is to use the fact that the transcription factor mutations that cause the commonest forms of MODY have extra-pancreatic manifestations not shared by type 1 or type 2 diabetes. Looking at hepatic genes regulated by HNF1A has led to the identification that highly-sensitive hepatic cell reactive protein (hsCRP) is lower in HNF1A-MODY than in all other kinds of diabetes and non-diabetic individuals. Using hsCRP to differentiate HNF1A-MODY from type 2 diabetes diagnosed before 45 years has a sensitivity and specificity both around 80% and an area under the receiver operated characteristic curve of >0.8, indicating a good discriminative test. A model including simple clinical characteristics can improve this further.

Simple pathophysiological features can also form the basis for selection for genetic testing e.g. presence of C-peptide indicating endogenous insulin secretion can be a marker to differentiate MODY cases from those assumed to have type 1 diabetes.

The results of a novel discovery experiment using metabolomics to identify putative MODY biomarkers will also be presented.

---

**S1.4**

**Genome-wide association studies for type 2 diabetes**

M Weedon
Exeter, UK.

Abstract unavailable.

---

**S2.1**

**Novel pathways and treatments in neuroendocrine tumours**

Mark Kidd & I Modlin
Department of Surgery, Yale University School of Medicine, New Haven, Connecticut, USA.

Neuroendocrine tumor cells express a diverse array of activating and inhibitory receptors. Each receptor transduces a signal via individual pathways which often interact or overlap. Common stimulatory receptor families include those for EGF/TGF<sub>α</sub>, FGF, IGF, PDGF, VEGF, and TGFβ. EGFR receptor (Her 1) activation results in several signal transduction cascades including Ras/Raf, MAPK, AKT and JNK leading to DNA synthesis and cell proliferation. Activated MAPK signaling is a common feature of GEP-NETs (‘carcinoids’), but the absence of activating mutations in the EGFR receptor suggests this is not due to EGFR/TGFβ. The four FGF receptors combine to create 48 isoforms; the predominant isoforms FGFR1-4 are expressed in all GEP-NETs; signaling activation is associated with mitogenesis and differentiation as well as cross-talk within the tumor microenvironment (endothelial cell activation). IGF1R is present in the majority (>70%) of GEP-NETs and is coupled to Ras/Raf, MAPK and the PI3K-AKT-mTOR pathway. IGFIR activation is associated with survival and proliferation in mitosis-competent cells (e.g. serotonin-mediated hepatocyte-production of IGFs by hepatic metastases) and hypertrophy in differentiated cells. PGRFs are occasionally expressed in GEP-NETs and are associated with MAPK/AKT activation. The majority of expression, however, occurs in tumor stroma, and is associated with angiogenesis. The VEGFR has also been identified in GEP-NETs, and the production of VEGF by NETs is coupled to endothelial cell mitogenesis/migration and angiogenesis. TGFβR are present in the majority (~99%) of GEP-NETs. In pancreatic NETs, they are coupled to the classical SMAD2/3 pathway and inhibition of proliferation. In contrast, in small intestinal NETs, TGFβR are coupled to SMAD4/7 which results in tumor proliferation via cross-activation of MAPK and AKT. Common inhibitory receptor families include those for TGFβ, interferon, somatostatin, dopamine and serotonin. Somatostatin receptors are coupled to PKA/CAMP and calcium channels and activation inhibits cell secretion. There is some evidence that proliferation may be diminished but the mechanisms have not been clearly delineated. Dopamine D2/D4 receptors activation may inhibit NET function, largely via cAMP inhibition, although there is considerable heterogeneity in dopamine receptor expression in tumors (D2 as well as stimulatory D1,3,5 – which activate PKA/CAMP). Interferons activate IFNγR1/2 and signal through JAK/STAT pathways leading to apoptosis. Certain subtypes of small intestinal NETs express serotonin receptors, usually 5-HT₂Bγ. Activation of these receptors inhibit serotonin secretion and cell proliferation via inhibition of

---

**Endocrine Abstracts (2011) Vol 25**
MAPK signaling. Many NETs also exhibit loss of the G1 checkpoint inhibitor P21WAF1/CIP1. Other potential signaling pathways include hedgehog (SHH – over-expressed in metastases) and NOTCH (potentially involved with secretion). In conclusion, the expression of a variety of receptors activating diverse transduction events that enable stimulation or inhibition of tumor growth provide a number of opportunities for therapeutic intervention that might target growth, secretion or angiogenesis.

S2.2
Integrated genome-wide DNA methylation and mRNA expression analysis of pancreatic NETs
Christina Thrilwell1,2, Laura Schulz2, Marianne Eymard2, Tim Meyer1,2, Brian Davidson1, Andrew Teschendorff2, Yan Jiao2, Tu-Vinh Luong1, Martyn Caplin1 & Stephan Beck2
1Royal Free Hospital Academic Unit for Neuroendocrine Tumours, Pond Street, London NW3 2QG, UK; 2UCL Cancer Institute, 72 Huntley Street, London WC1E 6BT, UK.

Integration of genetics and epigenetics has emerged as a powerful approach to study cellular differentiation and tumourigenesis. The study of DNA methylation is of particular importance in cancer as causal involvement has been demonstrated and it is the most stable of all epigenetic modifications, making it a desirable marker for both early detection and treatment of tumours. Hypermethylation of CpG sites in gene promoter regions leads to decreased gene expression, if such a gene is a tumour suppressor this leads to carcinogenesis.

Ten fresh frozen sporadic pancreatic NET liver tumours (3 low grade, 3 intermediate grade and 4 high grade) were analysed using the Illumina HumMeth27 beadarray (interrogating 27 500 CpG sites relating to 14 000 genes) and the Affymetrix HumanGene 1.0 ST mRNA expression array (which has 26 probes covering the full coding region of 28 869 genes).

Integrated DNA methylation and mRNA expression analysis comparing tumour grade in sporadic pancreatic NETs identified the HIF/p53 hypoxia pathway to be differentially activated between low and intermediate grade tumours (P=4×10\(^{-10}\)) and the Affymetrix HumanGene 1.0 ST mRNA expression array (which has 26 probes covering the full coding region of 28 869 genes).

This study has highlighted the significance of the HIF/p53 hypoxia pathway to be differentially activated between low and intermediate grade tumours (P=4×10\(^{-10}\)) and the Affymetrix HumanGene 1.0 ST mRNA expression array (which has 26 probes covering the full coding region of 28 869 genes).

These agents appear to be effective even when other treatments have failed and herald a new era in the management of patients with pancreatic NETs. Emerging evidence suggests that tyrosine kinase inhibitors may also be active in non-pancreatic NETs although mature data is awaited. Achieving an understanding of the how these agents fit into the treatment algorithm in relation to each other and alongside other treatment modalities as well in early-stage disease will necessitate numerous clinical studies and require significant collaboration amongst investigators.


S2.3
Tyrosine kinase inhibitors for neuroendocrine tumours
Juan W Valle
Department of Medical Oncology, The Christie NHS Foundation Trust, Wilmslow Road, Manchester, UK.

Neuroendocrine tumours (NETs) are a heterogeneous, uncommon tumours for whom there have been few practice-changing clinical studies. Sub-groups of patients may be defined by site of origin, histological sub-type, mitotic activity and evidence of disease progression. Chemotherapy is active in patients with advanced pancreatic NETs; however, benefits are modest and associated with significant (albeit manageable) toxicity. Additional treatment options are sparse. Two recently published studies have challenged our treatment paradigm in such patients.

Pancratic NETs are known to be vascular tumours with over-expression of vascular endothelial growth factor (VEGF, a key regulator in angiogenesis) and VEGFR receptor (VEGFR-2, VEGFR-3, platelet derived growth factor receptors-α and -β and c-kit). Continuous dosing of sunitinib, an oral multi-targeted tyrosine kinase inhibitor of this pathway, has been shown in a phase III, placebo-controlled study to significantly improve the progression-free survival (PFS, the primary end-point), objective response rate and overall survival in patients with well-differentiated, progressive pancreatic NETs with acceptable toxicity.

Another pathway over-stimulated in pancreatic NETs is the mammalian target of rapamycin (mTOR); stimulation of this serine-threonine kinase results in angiogenesis, cell growth and proliferation. Everolimus, an inhibitor of mTOR, has recently been shown to significantly prolong PFS in a placebo-controlled, phase III study in patients with progressive low- or intermediate-grade advanced pancreatic NET, once again with manageable toxicity.

S3.1
Can we make more brown adipose tissue to treat obesity?
W Van Marken Lichtenbelt
Maastricht University Medical Center, Maastricht, The Netherlands.

The incidence of the metabolic syndrome has reached epidemic levels in the western world. With respect to the energy balance most attention has been given to reducing energy (food) intake. Increasing energy expenditure is an important alternative strategy. Adaptive thermogenesis, which is the increase in energy expenditure in response to cold or diet, may be an effective way to affect the energy balance. Several studies have confirmed that humans show significant (mild) cold induced thermogenesis, i.e. without shivering. The individual variation in CIT is large. There are indications that CIT is reduced in obese subjects. Tissues shown to be involved in adults are skeletal muscle and brown adipose tissue (BAT). The most likely cellular mechanism in both tissues is mitochondrial uncoupling.

At the functional level, adipocytes can be subdivided into white and brown. The most important function of white adipocytes is energy storage, while the main function of brown adipocytes is heat production. Brown adipocytes are located in distinct BAT depots, in white adipose tissue (BRITe adipocytes), or in skeletal muscle tissue (BRUSCLE adipocytes).

Intriguingly, functional and active BAT is inversely correlated with age and body mass index (BMI) in humans. Indeed, thermogenic BAT is a major site for lipid breakdown and glucose uptake, and thus the thermogenic capacity of even small amounts of brown adipocytes has emerged as an attractive target for anti-diabesity therapies.

Animal and human studies indicate that recruitment and activation of brown and white adipocytes is heat production. Brown adipocytes are located in distinct BAT depots, in white adipose tissue (BRITe adipocytes), or in skeletal muscle tissue (BRUSCLE adipocytes). Intriguingly, functional and active BAT is inversely correlated with age and body mass index (BMI) in humans. Indeed, thermogenic BAT is a major site for lipid breakdown and glucose uptake, and thus the thermogenic capacity of even small amounts of brown adipocytes has emerged as an attractive target for anti-diabesity therapies.

S3.2
Familial partial lipodystrophy: an introduction and the importance of diagnosing FPLD
S Suliman
Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK.

Lipodystrophy including familial, acquired and secondary forms displays the full spectrum of the metabolic syndrome including severe insulin resistance, hyperinsulinemia, hypertension, hypertriglyceridaemia, low HDL cholesterol, and hyperglycaemia. This group have an absolute or partial reduction in fat mass,
yet often show more extreme metabolic features than obese individuals. Partial lipodystrophy may be genetic or acquired. Genetic causes of familial partial lipodystrophy (FPLD), often an autosomal dominant condition; include mutations in LMNA, PPARG, AKT2, ZMPSTE24, CD36C. However, the majority of subjects have no identified genetic etiology. Diagnosing FPLD has depended mainly on clinical observations of fatless limbs and phlebectasia (prominent leg veins). Using the Oxford Biobank (database of > 1000 healthy individuals in Oxfordshire) and PLD subjects from the Oxford Lipodystrophy Clinic, we have been able to define a normal ratio of central to peripheral skin-fold measurements in healthy individuals. This ratio is markedly increased (> 95%) in LMNA mutation positive FPLD subjects and subjects with clinical lipodystrophy but no known genetic etiology. Subjects with FPLD require individualised treatment in view of their adverse metabolic profile and increased cardiovascular risk. Specific treatments include leptin therapy, in leptin deficient subjects, which improves insulin resistance, hyperglycaemia and dyslipidaemia. Isolated reports suggest that thiazolidinediones have been successful although prospective studies have been less rewarding. Bariatric surgery has been rewarding in patients with FPLD. Familial partial lipodystrophy is less rare than previously reported with a high cardiovascular risk and require identification and individualised management.

References

$S3.3$

The link between insulin resistance, dyslipidaemia and fatty liver
Robert Semple
University of Cambridge, Cambridge, UK.

Insulin resistance (IR) is an important biochemical phenomenon because it is closely linked to major and highly prevalent diseases including diabetes mellitus, atherogenic dyslipidaemia, the fatty liver disease dysfuntion, and ovulatory dysfunction. Yet major barriers to understanding the mechanisms linking IR to these clinical diseases have included 1. the difficulty in discerning cause and effect relationships in associated phenomena in a complex multisytem disorder and 2. the limitation of conventional definitions of IR, which rely solely on impaired insulin action on glucose metabolism. Key analytical approaches to circumventing these problems include the creation of targeted genetic alterations in mice, and the study of naturally occurring single gene disorders of insulin action in humans. These complementary approaches over recent years have led to growing interest in the idea that fatty liver and metabolic dyslipidaemia only appear when IR is partial, affecting the hypoisulinemic actions of insulin without impeding insulin’s stimulatory effects on hepatic de novo lipogenesis. This means that compensatory hyperinsulinaemia can act through unaffected signalling pathways to drive liver fat accumulation and hypersecretion of hepatic VLDL. I shall critically review the evidence for this concept, focusing on metabolic studies in humans with rare single gene defects associated with severe IR.

$S3.4$

What you need to know about the genetics of hyperlipidaemia
Carol Shoulders, Claire Hutchinson, Emma Duncan & Rasheeta Sivapackianathan
Queen Mary University of London, London, UK.

This presentation will consider the ways that modern genetics and lipidomics are beginning to increase our understanding of specific lipid disorders that bear on human disease, ranging from life-threatening conditions of infancy through severe coronary heart disease of young adulthood, to indolent disorders of middle- and old-age. The case will be made for replacing the traditional, but now 45-year-old, Fredrickson and Levy ‘essentially phenotypic classification of hyperlipidaemia with one rising from genetic foundations.

Endocrine regulation of ageing

$S4.1$

The ageing process; somatotropic axis and calorie restriction
Andrzej Bartke & Michal Masternak
SIU School of Medicine, Springfield, Illinois, USA.

Mice with targeted deletion of GH receptor (GHRKO mice) are GH resistant, small, hypoinsulnemic, very sensitive to insulin and remarkably long-lived. We have previously reported that these animals failed to respond to calorie restriction (CR); by additional increase in insulin sensitivity or extension of longevity. Because GHRKO mice are characterized by increased adiposity and adiposity is normally associated with insulin resistance rather than sensitivity, we hypothesized that the impact of adipose tissue on metabolism must be profoundly altered in these animals. To test this hypothesis, we compared the effects of surgical removal of visceral (epidymidal and perinephric) fat in GHRKO and normal males. Visceral fat removal (VFR) enhanced insulin sensitivity (assessed by insulin levels and insulin and glucose tolerance tests) and reduced intramuscular fat content, body temperature and respiratory quotient in normal animals but had disparate, generally opposite effects on the same parameters in GHRKO mice. Moreover, VFR in GHRKO but not normal mice reduced circulating levels of adiponectin, which is known to promote insulin sensitivity and exert anti-inflamatory effects. Functional differences between VFR from normal and GHRKO mice implied by these findings were confirmed by measurements of lipolysis, adiponectin and interleukin-6 levels and expression of genes related to fat metabolism. We conclude that in the absence of GH signals, VFR acts to promote insulin sensitivity rather than resistance. Lack of beneficial effects of VFR on insulin signaling and longevity-associated traits in GHRKO mice may explain why CR, which drastically reduces adiposity, failed to improve insulin sensitivity or extend longevity in these animals. Supported by grants from the National Institute on Aging.

$S4.2$

The ageing process: insulin signalling and mTOR signalling
Colin Selman
University of Aberdeen, Aberdeen, UK.

Alterations in insulin/insulin-like growth factor 1 signalling (IIS) play a key role in lifespan extension in model organisms. For example, mice globally null for insulin receptor substrate protein 1 (IRS1), a major intracellular IIS effector, are both long-lived and have improved health during this long life. However, unlike long-lived GH dwarf mice and dietary restricted (DR) mice, IRS1 null mice combine an increased lifespan with lifelong insulin resistance. These findings suggest that enhanced insulin sensitivity is not a prerequisite for long-life. An additional signalling pathway that appears to plays a key role in longevity control is the target of rapamycin (TOR) pathway. Global deletion of ribosomal S6 protein kinase 1 (S6K1), a key effector of mTOR and IIS signalling, extends healthspan in female (but not male) mice. In addition, these mice are resistance to a range of age-related pathologies, including bone, immune and motor dysfunction. Unlike IRS1 nulls, S6K1 null mice were insulin sensitive in old age. S6K1 deletion also induced hepatic gene expression profiles similar to DR and IRS1 null mice, and muscle expression profiles of S6K1 null mice closely mimicked those of mice following AICAR treatment. Therefore, manipulation of mTOR (and AMPK) may mimic DR and may provide viable drug targets offering protection against diseases of aging.

$S4.3$

Cortisol, DHEAS and immunesenesence
Janet Lord, Anna Phillips & Wiebke Arlt
Birmingham University, Birmingham, UK.

Normal ageing is accompanied by increased organismal frailty, reflecting organ specific functional decline, with an associated increase in the likelihood of disease. The immune system undergoes significant decline with age, termed immunesenesence, which results in increased susceptibility to infection and reduced vaccination...
responses. Significant changes in the hormonal milieu also occur with age and it is clear that age-related changes in adrenal hormone secretion can impose a significant environmental effect on tissues in the ageing body, including the immune system. In humans the adrenopause results in a decline in the production of DHEA and DHEAS with age, while the production of cortisol is unaffected. Ageing is therefore accompanied by an increase in the cortisol:DHEAS ratio. The immune suppressive effect of cortisol is well established, but evidence is now increasing for an immune enhancing role for dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS). We have reported recently that DHEAS is able to enhance the response of neutrophils to bacterial (MLP, significantly increasing superoxide generation. Moreover we showed that neutrophils were unique amongst leukocytes in expressing a transporter for sulphate steroids such as DHEAS and that the effect on neutrophil superoxide generation was mediated via direct activation of protein kinase C. As neutrophils are the dominant leukocyte in the circulation and are the first defence against rapidly dividing bacteria, DHEAS may represent a significant neutrophil priming agent able to overcome the suppressive effects of cortisol.

Our previous data has also shown that at times of stress (both emotional and physical) in the older adult the cortisol:DHEAS ratio is further increased and is associated with suppression of neutrophil function and increased risk of infection. We therefore propose that the age-related changes in the HPA axis contribute to immunosenescence and that this effect is enhanced at times of stress in old age.

### S5.2

**Sonic hedgehog signalling and primary cilia in the adrenal**

Katy Cogger\(^1\), Leo Guasti\(^1\), Zahida Banu\(^1\), Phil Beales\(^1\), Alex Paul\(^2\), Ed Laufer\(^2\) & Peter King\(^3\)

\(^1\)WHRI, Queen Mary University of London, London, UK; \(^2\)Columbia University, New York, New York, USA; \(^3\)Institute of Child Health, London, UK.

The adrenogonadal primordium develops from a thickening of the coelomic epithelium covering the urogenital ridge. After segregation of the primordium into the bipotential gonad and the adrenocortical primordium, the cortex is encapsulated by mesenchymal cells and infiltrated by migrating sympathoadrenal cells, which form the medulla. We have shown that the cell fate regulator sonic hedgehog (Shh) is not required for the formation of the adrenocortical primordium but is required for its subsequent growth and development. Its expression is restricted to relatively undifferentiated cortical subcapsular cells and it signals to cells within the capsule. Lineage tracing studies in mice demonstrate that these signal receiving cells enter the cortex and adopt a steroidogenic phenotype, becoming zona glomerulosa and zona fasciculata cells, at least in part, via a Shh-expressing intermediate cell population.

The primary cillum is required for hedgehog (Hh) signalling, with disruption of genes required for cilia formation phenocopying Hh pathway mutations. Although there has been no report of ciliopathies associated with adrenal insufficiency to date, our studies have documented adrenal phenotypes in BBS 4 and 6 null mice consistent with impaired Shh signalling, and knockdown of IFT88 in the human adrenocortical carcinoma cell line H295R affects steroidogenesis. These data support a role for the primary cillum in adrenocortical differentiation, differentiation and function.

### S5.3

**The importance of primary cilia in adipogenic differentiation**

Vincent Marion

INSERM, Strasbourg, France.

Long considered as a vestige of evolution, the primary cillum has recently emerged as a crucial organelle in the regulation of cell function. Ciliated cells are ubiquitously present in the organism and until recently only two cellular types were considered as unciliated cells, namely the adipocyte and the hepatocyte. An in vitro approach evidenced that the pseudopodium was transiently ciliated during its terminal differentiation phase and that the primary cillum was acting as a detection sieve for anti-adipogenic signals as it carried both the Wnt receptor as well as the Sonic Hedgehog receptor, Smoothened. Inactivation of the chaperone-like BBS protein resulted in unciliated pseudocytes which were more prone to undergo terminal differentiation as well as massive triglyceride accumulation combined with higher leptin secretion; a situation found back when using dermal fibroblasts derived from BBS patients. BBS-associated obesity has been described to be of central origin, namely due to a leptin resistance as the hypothalamic arcuate nuclei were unable to sense plasma leptin. Our data indicate that the BBS-associated obesity could be of two origins, namely from a central hypothalamic dysfunction and from a more peripheral adipocyte-related one. Further in vitro studies on the adipocytes of our newly generated Bbs12 knock out mice and of the leptin resistant mouse, the Ob/Ob mouse, tend to confirm our previous in vivo findings that BBS-related obesity is not solely linked to a leptin resistant status.

#### S5.1

**Making sense: primary cilia in disease and development**

Phil Beales

UCL Institute of Child Health, London, UK.

Ubiquitous in nature, cilia and flagella comprise near identical structures with similar functions. The most obvious example of the latter is motility; driving movement of the organism or particle flow across the epithelial surface in fixed structures. In vertebrates, such motile cilia are evident in the respiratory epithelia, ependyma and oviducts. For over a century, non-motile cilia have been observed on the surface of most vertebrate cells but until recently their function has eluded us. Gathering evidence now points to critical roles for the mono-cilium in sensing the extracellular environment and perturbation gives rise to predictable panoply of clinical problems. I will review the evidence for cilia function during development and examine the consequences of cilia dysfunction in disease (ciliopathies).
S5.4 The molecular pathophysiology of the human obesity disorder, Bardet Biedl syndrome (BBS)
Val Sheffield
Department of Pediatrics and Howard Hughes Medical Institute, University of Iowa, Iowa, USA.

Bardet-Biedl syndrome (BBS) is a heterogeneous autosomal recessive disorder characterized by obesity, retinopathy, cognitive abnormalities, and polydactyly and other congenital anomalies. Patients also have an increased incidence of hypertension and diabetes. Mutations in at least 14 genes have been reported to independently cause BBS. In order to better understand the pathophysiology of BBS, we have generated BBS mouse models and have investigated the interaction of the protein products of the BBS genes. Seven BBS proteins form a stable protein complex known as the BBSome, which promotes cilium membrane elongation through Rab8. In addition, we have shown that a component of the BBSome (BBS1) interacts directly with the leptin receptor. Three additional BBS proteins have chaperonin homology and play a role in BBSome formation. BBS3, an ADP-ribosylation factor (ARF)-like small GTPase also known as ARL6, is not part of the BBSome complex and does not have chaperonin homology. We used Bbs3−/− mice to determine whether Bbs3 is required for BBSome formation. Loss of Bbs3 does not affect BBSome protein levels or BBSome formation. However, both the BBSome and Bbs3 localize to cilia, and loss of Bbs3 disrupts localization of BBSome proteins to cilia. Conversely, the BBSome is required for ciliary localization of Bbs3.

Many signaling proteins including G protein-coupled receptors localize to primary cilia, regulating cellular processes including differentiation, proliferation, organogenesis, and tumorigenesis. Bardet-Biedl syndrome (BBS) proteins are involved in maintaining ciliary function by mediating protein trafficking to the cilium.

The mechanisms governing ciliary trafficking by BBS proteins are not well understood. We show that a novel protein, Leucine-zipper transcription factor (LZTFL1), interacts with the BBSome, and regulates ciliary trafficking of this complex. We also show that all BBSome subunits are required for BBSome ciliary entry and that reduction of LZTFL1 restores BBSome ciliary trafficking in Bbs3 and BBS3-depleted cells. Our findings indicate that LZTFL1 is an important regulator of BBSome ciliary trafficking, and as such is a target for treatment.

S6.2 3-Iodothyronamine: a novel, biologically active thyroid hormone metabolite
Thomas Scanlan
Oregon Health and Science University, Portland, Oregon, USA.

We have recently discovered a novel, endogenous iodine containing aryl-ethanamine derivative that we believe is a metabolite of thyroxine, the major form of thyroid hormone produced in the thyroid gland of vertebrates. This compound is 3-iodothyronamine (T1AM), and it does not bind to or activate nuclear thyroid hormone receptors – the established target receptors of thyroid hormones – but instead functions as a potent agonist of trace amine associated receptor 1 (TAAR1), an orphan G protein-coupled receptor (GPCR), to rapidly modulate cAMP levels in cells expressing the GPCR. In addition, T1AM is an agonist of alpha-2a-adrenergic receptors and inhibits certain plasma membrane and vesicular monoamine transporters; however, it is not clear that any of these receptors or transporters are physiological targets of T1AM. The in vivo pharmacology of T1AM in many ways induces physiological changes that occur naturally in hibernating animals. Rodents treated with single pharmacological doses of T1AM display profound changes in thermal regulation, cardiac performance, and blood glucose homeostasis. Similar to the in vitro cell-based experiments, these in vivo effects occur with rapid kinetics suggesting that they are mediated by non-transcriptional mechanisms. In addition to displaying all of the above effects, hibernating rodents treated with single-dose T1AM demonstrate a fuel utilization preference away from carbohydrates and toward lipids. This presentation will focus on the pharmacological and physiological properties of T1AM and explore the potential therapeutic utility of this biogenic amine.

S6.3 The action of thyroid hormone in vascular tissues
Paul Davis1,2, Faith Davis1, Hung-Yun Lin1 & Shaker Mousa2
1Ordway Research Institute, Albany, New York, USA; 2Albany College of Pharmacy, Albany, New York, USA.

Iodothyronines stimulate neovascularization in tumor beds, brain, ischemic myocardium and striated muscle. The molecular basis of the action can be studied in models such as the chick choioallantoic membrane (CAM) and hamster microvascular endothelial cell (HDMEC) microtubule formation. Nongenomic and genomic actions of the hormone may contribute to angiogenesis. The initial description of the cell surface receptor for thyroid hormone (TRα and TRβ1, TRβ2) on integrin αvβ3 nongenomically linked thyroid hormone to angiogenesis via mitogen-activated protein kinase (MAPK; ERK1/2) and involved downstream transcription of basic fibroblast growth factor (bFGF), FVIIa and vascular endothelial growth factor (VEGF) genes. Further, crosstalk between the hormone receptor on the integrin and VEGF and bFGF receptors clustered with the integrin has also been demonstrated. Other pro-angiogenic hormone analogues are diiodothyropionic acid (DITPA) and GC-1. Tetraiodothyroacetic acid (tetrac) is an inhibitor of the actions of TRα and TRβ in the integrin and blocks thyroid hormone-induced angiogenesis. In the absence of TRα and TRβ, however, tetrac will also inhibit the actions of VEGF, platelet-derived growth factor (PDGF) and bFGF in CAM and HDMEC microtubule assays. Iodothyronines may also stimulate angiogenesis about areas of infarction in the myocardium in the intact experimental animal by a mechanism that may involve TRβ1. Hypoxia-inducible factor-1 (HIF-1) is a transcription factor important to ischemia-induced coronary artery collateralization; acting in cytoplasm, T3 induces expression of the HIF1α gene. Thus, thyroid hormone is pro-angiogenic by mechanisms that may begin at the plasma membrane, in cytoplasm or within the nucleus. Pro-angiogenic actions of the hormone initiated at the integrin may involve crosstalk at the cell surface with vascular growth factor receptors or, downstream, culminate in vascular growth factor gene expression. Acting at the integrin, tetrac is an anti-angiogenic by inhibiting actions of TRα and TRβ, and by blocking effects of VEGF, bFGF and PDGF.

Endocrine Abstracts (2011) Vol 25
The axons and terminals of GnRH neurons are closely apposed to and ensheathed by the tanycyte processes. Ultrastructural studies revealed that under conditions of low gonadotropin output such as in diestrus, GnRH-secreting nerve terminals are completely surrounded or engulfed by tanycytes, which prevent direct access to the portal vessels and thus create a diffusion barrier impeding GnRH entry into the pituitary portal circulation. During the preovulatory surge on the day of proestrus, a structural remodeling of tanycyes occurs, resulting in the release of the engulfl1ed axons and the establishment of a direct neurohaemal relationship between GnRH neuroendocrine neurons and the pituitary portal blood. These morphological changes presumably favor the release of GnRH into the portal vasculature; the lack of glia between the neuroendocrine terminals and the perivascular spaces results in the removal of a diffusion barrier, so that released neurohormones can reach the fenestrated capillaries of the median eminence more efficiently. Underlying signaling pathways responsible for these structural changes are comprised of highly diffusible gaseous molecules such as endothelial nitric oxide (NO) and paracrine communication processes involving receptors of the eNOS tyrosine kinase family, transforming growth factor β1 (TGFβ1) and eicosanoids such as prostaglandin-E2 (PGE2).

**S7.3 Food or sex: neuropeptides decide**
Iain Clarke
Department of Physiology, Monash University, Clayton, Australia.

As a general rule, hypothalamic neuropeptides that stimulate food intake act to inhibit the reproductive axis. We have studied the functions of two peptide systems in detail. Melanocortins, such as melanocyte stimulating hormone-α, β and γ, are products of the pro-opiomelanocortin (POMC) gene, produced in cells of the arcuate nucleus and act to inhibit feeding. Melanocortins also stimulate the reproductive axis. In lean hypogonadotropic ovariectomised ewes, POMC gene expression is reduced, but i.e.v. infusion of lepim increases expression of this gene and peptide levels as well as restoring pulsatile LH secretion. i.e.v. infusion of a melanocortin receptor agonist also increases LH secretion in lean animals, suggesting that the melanocortin system may be the means by which the reproductive axis is regulated by changing metabolic state. Furthermore, the agonist can stimulate pulsatile LH secretion in the luteal phase of the estrous cycle. Melanocortin cells have reciprocal communication with kisspeptin cells of the arcuate nucleus, so effects of these two systems on the GnRH cells may be integrated. Gonadotropin inhibitory hormone (GnIH) is produced by cells of the dorsomedial hypothalamic nucleus/paraventricular nucleus. These cells project to GnRH cells and appetite regulating cells as well as to the neurosecretory zone of the median eminence. In sheep at least, GnIH acts to inhibit both GnRH cells and pituitary gonadotropes. The peptide also has a potent effect to stimulate food intake. GnIH gene expression is reduced in the periovulatory period, consistent with data from other species showing a reduction in appetite at the time of ovulation. Thus, GnIH signals to inhibit reproduction and stimulate food intake.

The melanocortins and GnIH both act to regulate reproduction and food intake. Reduced melanocortin levels in lean condition may be the cause of lowered activity of the reproductive axis.

**S7.4 A role for kisspeptin/neurokinin B/dynorphin neurons in initiating and maintaining reproduction**
Victor Navarro1,2
1University of Washington, Seattle, Washington, USA; 2University of Cordoba, Cordoba, Spain.

Puberty is a tightly regulated process through which an individual attains reproductive capability. An intricate network of central and peripheral factors play a role in this process; however, the cellular and molecular events that initiate and sustain adult reproductive function remain largely unknown. Recently, kisspeptin (encoded by Kiss1) and neurokinin B (NKB, encoded by TAC3 in humans and Tac2 in rodents) have been shown to be gate-keepers of puberty onset. Studies in humans and rodents have shown loss-of-function mutations in either Kiss1/NKB or their receptors, Kiss1r/NK3R, produce congenital hypogonadotropic hypogonadism in the pituitary–gonadal axis. The expression and correlations that link sexual development to changes in Kiss1 and NKB expression, we have little understanding of precisely how NKB and kisspeptin
guide pubertal maturation. We and others have shown that kisspeptin, NKB and dynorphin A (Dyn) are co-expressed in neurons of the arcuate nucleus; moreover, these neurons also co-express NK3R. NKB has been shown to stimulate LH release, presumably by acting autocrinally on these Kiss1/NK3/Dyn neurons to induce kisspeptin-mediated GnRH secretion. We have proposed a model in which NKB works in concert with the counter-regulatory action of dyn (acting through interneurons) to shape the coordinated ultradian release of kisspeptin and GnRH and thereby drive the pulsatile release of LH secretion that is essential for the onset of puberty and for the maintenance of reproductive function in the adult.

Hormones and bone metabolism

S8.1

Fracture risk in patients with diabetes
L Hofbauer
Dresden, Germany.
Abstract unavailable.

S8.2

Muscle weakness, falls and vitamin D deficiency

Abstract unavailable.

S8.3

Effects of male hypogonadism on bone metabolism
Jean-Marc Kaufman
Ghent University Hospital, Gent, Belgium.
Pubertal exposure to rising blood concentrations of sex steroids, partly in concert with transiently increased activity of the somatotropic axis, is instrumental in a pubertal acceleration of bone growth and acquisition of bone mass, followed by growth inhibition and closure of the epiphyseal cartilages in late puberty. As is pubertal acceleration of bone growth and acquisition of bone mass, followed by a result, many of these individuals are at increased risk of osteoporosis, largely because of the endocrine changes induced by treatment characterised by a reduction in the level of bioavailable oestradiol. The associated increase in bone turnover that accompanies cancer treatment induced bone loss (CTIBL) can lead to a 40–50% increase in the rate of fragility fractures. The use of bisphosphonates in early cancer has become increasingly important to prevent adverse effects of cancer treatments on bone health. These include chemotheraphy induced ovarian failure and the use of aromatase inhibitors. Bisphosphonate strategies, similar to those used to treat post-menopausal osteoporosis, are the intervention of choice for patients with low bone mineral density (BMD) or rapid bone loss, along with adequate calcium and vitamin D intake and a healthy lifestyle. The results of several intervention studies with zoledronic acid in early breast cancer indicate that BMD is maintained and increased bone turnover normalised. There are also preliminary data from smaller studies using oral bisphosphonates at standard osteoporosis doses, as well as the new bone targeted agent denosumab, an antibody to RANKL. In addition to the beneficial effects of bisphosphonates on bone health, some data from trials in early breast cancer suggest that they may also reduce cancer recurrence rates in the adjuvant setting. Guidelines on the management of CTIBL have recently been formulated and utilise a risk adapted strategy similar to that used in the management of postmenopausal osteoporosis, with the exception that the recommended BMD threshold for intervention with pharmacological treatments is somewhat higher due to the accelerated rate of bone loss.

Doping: performance enhancing substances in sport and their detection

S9.1

Anabolic steroids in the gym
A Kicman
Department of Forensic Science and Drug Monitoring, King’s College, Drug Control Centre, London, UK.
Bodybuilders and athletes have recognised for several decades that the use of anabolic steroids can promote muscle growth and strength but it is only relatively recently that these agents are being revisited for clinical purposes. The pharmacology of anabolic steroids is not well understood, although intracellular steroid metabolism and also the topology of the bound androgen receptor interacting with co-activators are considered to be important factors. Behavioural changes by genomic and non-genomic pathways probably help motivate training. Doping with anabolic steroids can result in damage to health but it is important for endocrinologists not to exaggerate the risks but to emphasise to users that an attitude of personal invulnerability to their adverse effects is certainly misguided. Despite the large number of xenobiotic anabolic steroids available, testosterone continues to be the most common adverse finding in drug control tests undertaken by World Anti-doping Agency accredited laboratories. The detection of testosterone administration, nonetheless, remains challenging and a number of analytical approaches are now advocated, including using the individual as his own reference, a so-called athlete’s biological passport, and determination of steroid carbon-isotope ratio signatures.

S9.2

Compounds enhancing oxygen delivery
Peter Hennemutha1,2
1Norwegian Doping Control Laboratory, Oslo University Hospital, Oslo, Norway; 2School of Pharmacy, University of Oslo, Oslo, Norway.

For more than 25 years substances and methods enhancing oxygen delivery in the body have been of great concern in the fight against doping. The clear influence of an increased amount of red blood cells on physical performance, most obvious for endurance sports, has alerted the anti-doping authorities to put a high priority on efforts to disclose the administration of such doping practices.
The annually updated WADA-prohibited list mentions several erythropoiesis stimulating agents (ESA) as well as methods for the enhancement of oxygen transfer. The indicated substances include all different glycoprotein forms of erythropoietin preparations, synthetic peptidic compounds (Hematide/Peginesatide) as well as small molecular hypoxia-inducible factor (HIF) stabilizers. Prohibited methods like autologous and non-autologous blood transfusions are as well prohibited as haemoglobin-based oxygen carriers (HBOCs). The analytical approaches to disclose these kinds of doping range from direct detection of the actual prohibited substance or its markers (metabolites) to indirect detection methods monitoring the effects of those doping practices through the establishment of the Athlete’s Biological Passport. A broad selection of analytical techniques has been developed including among others chromatographic separation with mass spectrometric detection (LC–MS), isoelectric focusing (IEF) and flow cytometry. For several of the discussed substances a possible performance enhancing effect lasts considerably longer than its presence in the body. Therefore, doping control measures have been extended by an indirect approach, monitoring an individual haematological profile over time documented as the Athlete’s blood passport. Comprehensive harmonized guidelines have been established through the world anti-doping agency (WADA) in order to describe pre-analytical, analytical and interpretational criteria for its application in doping control.

S9.3
Beyond reasonable doubt: catching the GH cheats
R Holt
University of Southampton, Southampton, UK.

There is widespread anecdotal evidence that GH has been misused by athletes for its anabolic and lipolytic properties since the early 1980s, at least a decade before GH was used therapeutically by adult endocrinologists. Since then a number of high profile athletes have admitted using GH. Despite its widespread abuse, there is debate about whether GH is ergogenic. Until recently most scientific studies have not shown a performance enhancing effect but most have employed an inappropriate design to show a benefit. Although GH is on the World Anti-doping Agency (WADA) list of banned substances, the detection of abuse with GH is challenging. Two approaches have been developed; the first is based on the measurement of pituitary GH isoforms and was introduced at the Athens Olympic Games in 2004. The first analytical adverse finding using this test was made in February 2010 following an out-of-competition blood sample. The second approach is based on the measurement of IGF1 and N-terminal pro-peptide of type III collagen, both of which are markers of GH action. Both markers rise in a dose dependent manner and are largely unaffected by other regulators of GH secretion. When combined with gender specific discriminant function analysis, they achieve a greater sensitivity and longer window of detection than the isoform test. The development of this test is nearing completion.

S9.4
Beyond reasonable doubt: catching the GH cheats
Richard Holt
Faculty of Medicine, University of Southampton, Southampton, UK.

There is widespread anecdotal evidence that GH has been misused by athletes for its anabolic and lipolytic properties since the early 1980s, at least a decade before GH was used therapeutically by adult endocrinologists. Since then a number of high profile athletes have admitted using GH. Despite its widespread abuse, there is debate about whether GH is ergogenic. Until recently most scientific studies have not shown a performance enhancing effect but most have employed an inappropriate design to show a benefit. Although GH is on the world anti-doping agency (WADA) list of banned substances, the detection of abuse with GH is challenging. Two approaches have been developed; the first is based on the measurement of pituitary GH isoforms and was introduced at the Athens Olympic Games in 2004. The first analytical adverse finding using this test was made in February 2010 following an out-of-competition blood sample. The second approach is based on the measurement of IGF1 and N-terminal pro-peptide of type III collagen, both of which are markers of GH action. Both markers rise in a dose dependent manner and are largely unaffected by other regulators of GH secretion. When combined with gender specific discriminant function analysis, they achieve a greater sensitivity and longer window of detection than the isoform test. The development of this test is nearing completion.

Endocrine Abstracts (2011) Vol 25
Clinical Management Workshops
Generously supported by Clinical Endocrinology
The management of difficult Graves’ disease

CM1.1

Autoimmune hyperthyroidism: a spectrum of causes
S Pearce1,2
1 Institute of Human Genetics, International Centre for Life, Newcastle University, Newcastle upon Tyne NE1 3BZ, UK; 2Endocrine Unit, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, UK.

Autoimmune hyperthyroidism has a complex aetiology including both environmental and inherited components. Amongst the environmental factors that are well documented to be important are smoking and stressful life events. The prevalence of all forms of autoimmune hyperthyroidism is known to be higher in women, but the effects of oestrogen are complex: the combined oral contraceptive pill protecting against Graves’ disease, whereas pregnancy predisposes. Autoimmune hyperthyroidism also occurs in rare individuals during recovery from an immunosuppressed state due to HIV infection or following allogemnathan treatment. Genomic factors that are well documented include predisposing alleles at the MHC, CTLA4 and PTPN22 loci. Other candidate genes that have a role include TSHR. Recent genome-wide linkage studies have also shown several other loci with weak effects that appear to contribute, including PTPN22 and CD226.

CM1.2

Thyroid storm, aranulocytosis, vasculitis, hepatitis: what to do next?
A Allahabadia
Sheffield, UK.

Abstract unavailable.

CM1.3

The management of Graves’ ophthalmopathy and dermopathy
Luigi Bartalena
University of Insubria, Varese, Italy.

Extrathyroidal manifestations of Graves’ disease include eye disease (Graves’ ophthalmopathy, GO), dermopathy (also known as pretibial myxedema) and nail changes (acropachy). While about 30% of Graves’ patients are affected with mild to moderately severe GO, dermopathy is present only in 1–4% of cases, and acropachy in 0.1–0.4%. Management of GO represents a complex and unresolved problem, and the treatment outcome is frequently unsatisfactory both to the patient and the physician. Medical treatment is often followed by rehabilitative surgery, including orbital decompression, squint surgery, eyelid surgery. An aggressive medical treatment is indicated only when GO is active, i.e. in the inflammatory phase of the disease. Mild active GO usually does not require a specific treatment but only local measures such as artificial tears, ointments, dark lenses, prisms (for mild diplopia), and general measures, such as refrain from smoking. If however the quality of life is severely affected, the patient can be admitted to active treatment as for moderate-to-severe cases. First-line treatment for moderate-to-severe and active GO is represented by glucocorticoids. The latter can be administered via the oral route or the i.v. route. Randomized clinical trials have shown that the i.v. route is more effective and, in general, better tolerated. Severe side effects may however occur. Accordingly, this treatment should preferably be performed in specialized centers under strict surveillance. There is no evidence as to the optimal regimen. A commonly used schedule consists of 12 weekly slow infusions, with a cumulative dose of 4.5–5 g methylprednisolone. Some evidence suggests that a cumulative dose <8 g per cycle is associated with a low risk of hepatotoxicity. Unfortunately, treatment outcome is not always favourable. If response is not satisfactory and GO is still active, treatment options include a second course of i.v. glucocorticoids associated with orbital radiotherapy, or oral glucocorticoids combined with cyclosporine. A promising drug for GO (and Graves’ hyperthyroidism) is rituximab, but randomized clinical trials on the use of this drug are lacking. In cases of sight-threatening GO (mostly due to dysthyroid optic neuropathy), i.v. methylprednisolone at very high doses (1 g for three consecutive days, to be repeated on the next week) is the first-line treatment. If the response is absent or poor within 2 weeks, the patient should be promptly submitted to orbital decompression. Rehabilitative surgery, if needed, should be performed after GO has been inactive for at least 6 months. If more than one operation is required, orbital decompression, squint surgery and eyelid surgery should be performed in this sequence.

CM1.4

New therapies for Graves’ disease
Colin Dayan
Cardiff University, Cardiff, UK.

While the aetiology of Graves’ disease is now well-established, treatment for Graves’ disease has largely remained unchanged for 50 years. However, relapse rates following antithyroid drug treatment remain high, destructive therapy to the thyroid is not without its short and long-term risks and there have been limited studies of the benefits of different treatments in terms of long-term outcomes such as cardiovascular disease. There therefore remains much room for optimisation of our therapeutic approach. The advent of second generation assays for TSH receptor antibodies offers the possibility of targeted timing of the duration of antithyroid drug therapy and recent studies have improved predictive scores and identified additional factors such as serum selenium levels and common genetic variation that may modify the response to treatment. Prior thionamide therapy and the use steroids also modulate the response to radioiodine therapy. The duration of exposure to high thyroid hormone levels may impact on long-term cardiovascular and osteoporotic outcomes and new data is gradually becoming available that may influence the selection of therapy for Graves’ disease. B-cell therapy has recently been tried for Graves’ disease and Graves orbitopathy and may improve remissions rates after anti thyroid drug therapy. New developments in blocking antibody-TSH receptor monoclonal antibodies and the design of small molecule inhibitors of the TSH receptor may allow for more rapid induction of remission and improved outcomes especially in refractory disease.

Endocrine problems in pregnancy

CM2.1

Hypothyroidism and euthyroid antibody-positivity in pregnancy
S Chan
Department of Obstetrics and Gynaecology, Birmingham Women’s Hospital, Birmingham, UK.

Overt hypothyroidism, diagnosed and treated with l-thyroxine pre-pregnancy, affects 1% of pregnancies. Untreated maternal hypothyroidism is associated with adverse obstetric and neonatal outcomes, and even when partially treated the risks of miscarriage and prematurity remain elevated compared to euthyroidism. With increased understanding of the normal physiological changes to thyroid function during pregnancy, there should be a predictive approach to thyroxine dose adjustments in order to prevent the development of abnormalities in thyroid function, which could impact upon fetoplacental development. The vast majority of women will require a 30–50% dose increase by 5–8 weeks of gestation. Gestation specific reference ranges should be used, and these vary between assay platforms and patient populations. Since subclinical hypothyroidism is not associated with any adverse obstetric outcomes, and given that even within the normal reference range there are correlations between serum TSH and free T4 concentrations with perinatal loss and fetal malposition, the aim of treatment should be to maintain TSH within the lower part and free T4 within the upper part of the respective normal reference ranges. The roles of universal screening for and treatment of previously undiagnosed subclinical hypothyroidism (defined as elevated serum TSH accompanied by normal free T4; 2.5% of pregnancies) and isolated hypothyroxinaemia (normal serum TSH accompanied by low free T4; 2% of pregnancies) in pregnancy remain controversial. Although these biochemical abnormalities have been associated with increased obstetric risks and impaired offspring neurodevelopment in some
Two small clinical trials have suggested that L-thyroxine treatment could halve euthyroid, they are also at increased risk of miscarriage and preterm delivery. Despite the majority being biochemically euthyroid, they are also at increased risk of miscarriage and preterm delivery. Two small clinical trials have suggested that L-thyroxine treatment could halve these risks. Our group in Birmingham has just received EME-funding to commence a multi-centred (21 UK centres) randomised controlled trial (TABLET) to control maternal hyperthyroidism in a substantially larger group of women. Two key issues which remain to be answered are i) whether screening for subclinical thyroid dysfunction and thyroid autoimmunity, and the commencement of L-thyroxine treatment PRECONCEPTION could normalise obstetric outcomes and offspring neurodevelopment, and ii) whether there are specific subgroups of women who would definitely benefit from L-thyroxine treatment.

**CM2.2**

**Treatment of hyperthyroidism in pregnancy: risk for the unborn**
Maurizio Clementi, Matteo Cassina & Elena Di Gianantonio
Clinical Genetics - University, Padua, Italy.

Clinical hyperthyroidism is not uncommon in pregnancy, with a reported prevalence of 0.1–0.4%, and is caused most frequently by Graves disease. Careful monitoring of thyroid function is critical in preventing the many potential complications that can occur in pregnancies of mothers with hyperthyroidism. Maternal complications include hypertension, thyroid storm, heart failure preterm labour, and placental abruption. Foetal and neonatal complications include stillbirth, intrauterine growth restriction, low birth weight, heart failure, hyperthyroidism, and hypothyroidism with or without goitre. Currently, the drug of choice to control maternal hyperthyroidism is propylthiouracil (PTU) and methimazole (MMI)/carbamazepine (CZ). Both medications cross the human placenta with relatively similar transfer and placental clearance rates. PTU and MMDCZ are equivalent in terms of their efficacy in the treatment of clinical hyperthyroidism. Some reports suggested an association between specific congenital malformations (MMI embryopathy) and prenatal exposure to MMI. Using prospective data from the European Teratogen Information Services, Di Gianantonio concluded that there might be a higher than expected incidence of choanal and oesophageal atresia in foetuses exposed to MMI between the third and seventh weeks of gestation. Further reports of congenital anomalies, specifically of choanal atresia, have definitely suggested that MMI may be a new teratogen. For PTU the association with possible teratogenic risk is unclear, but this difference could be an artifact due to the lack of studies on PTU. However, recent studies failed to detect a significant association between PTU exposure during the first trimester of pregnancy and congenital malformations. In view of these reports, it is generally recommended that, when available, PTU be preferred as the initial therapy for maternal hyperthyroidism during the first trimester of pregnancy. However, the prescription of PTU later in pregnancy and during lactation should take into account the reported risk of hepatotoxicity in both the mother and the child.

**CM2.3**

**Management of adrenal disorders in pregnancy**
Wiebke Arlt
Centre for Endocrinology, Diabetes and Metabolism, University of Birmingham, Birmingham, UK.

The manifestation of adrenal disorders during pregnancy is a rare but regularly occurring event and can pose significant problems for the involved medical professionals. This lecture will give an overview of the major management issues surrounding disorders of the adrenal cortex during pregnancy. This includes the requirements for the adjustment of corticosteroid replacement therapy in adrenal insufficiency, due to the physiological changes in corticosteroid secretion during pregnancy and subsequent to the anti-mineralocorticoid action of progesterone. The lecture will include brief remarks on the management of the pregnant patient with congenital adrenal hyperplasia. Cushing syndrome can present during pregnancy and the lecture will highlight the diagnostic and therapeutic problems associated with this, including the choice and timing of medical and surgical interventions. Finally, major issues associated with the management of adrenal masses during pregnancy will be discussed.

**CM2.4**

**Pituitary disease in pregnancy (including NFPA, prolactinomas, DI)**
J Lenders
Nijmegen, The Netherlands.

Abstract unavailable.

**CM3.1**

**Focused radiotherapy as primary treatment for pituitary disease**
M Brada
The Institute of Cancer Research, London, UK.

Fractionated radiotherapy (RT) is effective in achieving disease control and normalisation of hormone levels. While overall safe, it is not devoid of side effects and should only be employed when the risks from the disease are considered to outweigh the risks from treatment. Currently RT tends to be withheld until progression unless there is a threat to function, particularly vision, from progressive tumour. RT is recommended for patients with secreting adenoma not achieving biochemical cure following surgery and medical treatment. Normalisation takes months to years and the delay is related to pre-treatment hormone levels.

Modern developments in radiotherapy aim to treat less normal tissue to significant radiation doses minimising the risk of late normal tissue injury. Treatment can be given as fractionated (fractionated stereotactic radiotherapy – fSRT/SCRT) or single fraction treatment (stereotactic radiosurgery – SRS) and relies on increased accuracy of tumour delineation with MRI. While there is perception that it is the use of modern technology which determines the outcome, the success is more likely related to operator skill and expertise and the accuracy in identifying the tumour.

Conventional fractionated RT achieves tumour control in 90–95% of patients at 10 and 85–90% at 20 years. Published results of ISRT show similar early results but have not reached the maturity of long term results of conventional RT. SRS, while apparently more convenient, is less effective in achieving tumour control without faster decline in hormone levels in secreting tumours. SRS of larger adenomas close to critical structures carries a significant risk of radiation damage. Fractionated irradiation either as modern conformal or fractionated stereotactic RT remains the standard of care with SRS considered as an experimental and in some instances less effective treatment.

**CM3.2**

**Focused radiotherapy as primary treatment for pituitary disease**
J Rowe
Shrewsbury, UK.

Abstract unavailable.

**CM3.3**

**Focused radiotherapy as a salvage treatment for pituitary disease**
F Yards
Norfolk and Norwich University Hospital, Norwich, UK.

There have been enormous advances in the medical and surgical management of pituitary tumours in recent years. Stereotactic radiotherapy has also allowed the...
use of more focused fractionated radiotherapy in the hope of minimising bystander damage and the long term sequelae of radiotherapy to the surrounding normal brain tissue.

With these advances, the number of patients requiring salvage treatment of any kind is reducing, and concerns persist about the long term safety of all forms of radiotherapy. However, there remains a small cohort of patients with refractory or recurrent disease despite all conventional treatments. This presentation will include the latest data on the use of focused radiotherapy or radiosurgery to such difficult cases, following surgery and fractionated radiotherapy. The largest group of patients treated with focused radiotherapy as a salvage treatment is acromegaly. This presentation will therefore concentrate on the efficacy of the treatment in this patient group, as well as reviewing both published and unpublished data from other tumours. Such heavily pre-treated cases also have a high incidence of pituitary failure: 63% in one series. We will present data to suggest that further loss of pituitary function is common after the application of focused radiotherapy – occurring in 66% of the remaining cases by 2 years, although in our series, no other adverse events have been recorded to date. The presentation will therefore discuss the safety data from both published and unpublished series in detail, and attempt to establish the best subset of patients in whom to consider this treatment modality.

**CM4.1**

The clinical spectrum of DSD

Ieuan Hughes

University of Cambridge, Cambridge, UK.

The variability in the manifestation of DSD covers a spectrum ranging from normal external female and male phenotypes to ambiguous genitalia. The latter scenario represents the *sino qua non* of DSD and poses a fundamental problem at birth – the inability to sex assign instantaneously. The typical newborn phenotype is represented by the ambiguity of a small penis/enlarged clitoris, labioscrotal folds, a single perineal orifice and gonads which may, or may not, be palpable. The paradigm of ‘true’ ambiguous genitalia of the newborn is congenital adrenal hyperplasia. This disorder needs to be confirmed or not, early, in view of its life-threatening risk. The diagnosis should be straightforward. A similar phenotype, but associated with an XY karyotype, requires a more complex investigation strategy which may not result in a definitive diagnosis. The boundaries of the spectrum can include conditions as common as isolated hypospadias, undescended testes, and isolated labial fusion or as rare as complete androgen insensitivity syndrome. Whatever the underlying cause, investigators must approach the problem from a thorough understanding of the genetic and hormonal control of normal fetal sex development.

**CM4.2**

Management of disorders of sex development (DSD) across the lifespan: presentation in adolescence

Olaf Hiort

University of Lübeck, Lübeck, Germany.

Despite recent enormous advances in the understanding of the molecular mechanisms of sex development, the medical decisions made in patients with disorders of sex development (DSD) are mostly lacking evidence-based principles and are carried on case by case evaluations. Very critical discussions have focused on approaches on gender assignment and treatment of DSD. Any decision should be based on a correct diagnosis and possible prediction of development during puberty and adulthood of the affected individual. Puberty plays a pivotal role not only because of the physical changes induced by endogenous or supplementary steroids, but also as a time for sexual orientation and promotion of gender identity. Therefore, many aspects from psychosocial guidance to medical treatment including hormone supplementation and surgical procedures have to be considered. The patient should be fully aware of the underlying condition and should be able to reflect any therapeutically valid options. This can only be managed by a multidisciplinary team involving many subspecialties. Most of all, experienced professionalism is needed to deal with these patients.

**CM4.3**

Turner/Klinefelter/Noonan syndrome: case presentations

Gerard Conway

UCLH Foundation Trust, London, UK.

Women with a 46,XY karyotype comprise a heterogeneous group who differ not only in their diagnostic category and anatomy but also in their journey from paediatric to adult services. Transition care should be an individualised process covering past experiences, current medical and surgical needs and future prospects is required for optimal wellbeing. A multidisciplinary team is helpful in providing this care and liaison with supports groups (for instance the ANSSG) is essential in order to keep informed of current issues in this area.

In a prospective audit of the clinical indications for referral and on-going clinical needs for girls aged 12–20 years seen in a specialist DSD clinic over a 6-month period we used a simple ‘traffic light’ classification: green for low, amber for moderate, and red for high. Fifty girls were seen during the study period and all were referred from paediatric services. Patients may have had one or more indication(s) for referral to the adult clinic and these were: urology/gynaecology (70%), endocrinology (42%) and psychology (14%). The most common indication for on-going clinical input was psychology, with 46% of patients requiring monitoring and intervention. Of the 14 patients (28%) classified red suggesting that they had an urgent clinical need, psychology was a major factor in all but one patient.

Despite progress in the understanding the genetic basis of human sexual development a specific molecular diagnosis is identified in only in a small percentage of cases of DSD presenting to an adults DSD clinic. Many previous diagnoses made on clinical grounds are found to be inaccurate. This is especially true in older patients who have had their diagnosis many years previously. It is well recognised that a delayed recognition of the condition can lead to greater difficulties in accepting the diagnosis. Access to specialist laboratory services is required to make an accurate diagnosis after gonadectomy.

**CM4.4**

Radiotherapy, cognition and cerebrovascular disease: what is the evidence?

John Ayuk

University Hospital Birmingham, Birmingham, UK.

Contemporary management of pituitary tumours is based on a multidisciplinary approach involving surgery, radiotherapy (RT) and medical therapy. External beam RT significantly reduces the likelihood of tumour re-growth following surgery for non-functioning pituitary adenomas and suppresses hyperecretion in hormonally-active tumours. However, over the years, a number of potentially significant complications of pituitary RT have been described. Radiation may cause a variety of vascular injuries and haemodynamic changes to the cerebral vasculature, and several authors have reported cerebrovascular complications and an increase in cerebrovascular mortality in patients receiving RT for pituitary and other central nervous system tumours. It is also possible that pituitary RT increases the risk of cerebrovascular disease by causing hypopituitarism, which is itself associated with an increase in vascular mortality. However, questions remain with regards to causation. Patients with pituitary tumours frequently report problems with memory and sustained attention that impact upon normal daily activities. During conventional pituitary RT, the limbic system, the hippocampus, the mammillary bodies and the pre-frontal cortex all receive a significant amount of radiation. It has been suggested that pituitary RT may be associated with cognitive impairment, but at present it is unclear whether any causal link exists between pituitary RT and abnormalities of memory and higher mental function.

Stereotactic radiosurgery delivers multiple low-energy beams toward a target with improved stereotactic accuracy. The principal advantage is that it reduces the dose of radiation received bytransirradiated tissue close to the target. It is a relatively new technique, only recently introduced in the management of pituitary disease. Some authors have suggested the ability to accurately direct high doses of radiation to the pituitary area with relative sparing of the surrounding tissues results in a more beneficial adverse profile. However, further data are required to fully assess the long-term adverse profile of stereotactic radiosurgery.
New approaches to molecular diagnosis
John Achermann
UCL Institute of Child Health, University College London, London, UK.

It is now around 20 years since the identification of SRY as a primary testis-determining gene and the molecular characterisation of many of the key enzymes and receptors involved in androgen synthesis and action. Although significant progress has been made since then in identifying other components involved in sex development, we are still unable to find an underlying genetic cause in many individuals with these conditions. Efforts to identify specific genetic causes of DSD are impeded by several factors such as phenotypic variability, the lack of specific biochemical markers and the high frequency of ‘unusual’ genetic mechanisms such as sex-limited dominant inheritance or de-novo events. Indeed, as DSD is usually associated with infertility, classic large pedigrees amenable to linkage or mapping studies are relatively rare. Traditionally, a candidate gene approach has been used to identify many cases of DSD, which can be time consuming and costly. However, the development of new nano-technologies means that we could potentially identify the genetic causes of DSD on a high-throughput scale, and can start to address some newer genetic mechanisms that might be important in influencing disease phenotype (e.g. digenic or oligogenic inheritance, gene dosage, epigenetic regulation). Here, I will provide an overview of new approaches to: i) karyotyping and the assessment of copy number variation (e.g. array CGH, SNP-based technologies); ii) high-throughput sequencing (such as ‘resequencing’ microarrays and next generation sequencing technologies); iii) determining protein or methylation ‘signatures’. The challenges will be cost, scale, organisation/collaboration, bioinformatics, bioethics, predicting in vivo functional effects, and integrating this wealth of information into an appropriate systems model relevant to DSD and of positive benefit to individuals with these conditions.
Applied Physiology Workshop
Seeing is believing – cutting edge in vivo cell imaging

AP1.1
Cellular imaging in the pituitary: in vivo confocal microscopy
P Mollard
Department of Endocrinology, Institute of Functional Genomics, Centre National de la Recherche Scientifique (CNRS), University of Montpellier, Montpellier, France.

Understanding the dynamic control of hormonal and metabolic homeostasis requires a description of the input, secretory and output mechanisms that underlie the life cycle of hormone pulses. Whilst input stimuli and hormone output have been measured, in vivo measurements of the blood microcirculation during a secretary pulse, and signalling at the cell and population level in the intact organ have not. To achieve this long-standing question, we recently developed a strategy to image in real-time the life cycle of hormone pulses in the pituitary gland by using a new imaging approach utilising long range objectives (LaFont et al. PNAS 2010 107: 4465–4470). It enabled us to measure local blood flow, oxygen partial pressure and cell activity at single-cell resolution in mouse pituitary glands in situ. When secretagogue (GHRH) distribution was modelled with fluorescent markers injected into either the bloodstream or the nearby intercapillary space, a restricted distribution gradient evolved within the pituitary parenchyma. Injection of GHRH led to stimulation of both GIH cell network activities and GH secretion, which was temporally associated with increases in blood flow rates and oxygen supply by capillaries, as well as oxygen consumption. Moreover, we observed a time-limiting step for hormone output at the perivascular level; macromolecules injected into the extracellulare parenchyma moved rapidly to the perivascular space, but were then cleared more slowly in a size-dependent manner into capillary blood. Hence, GH pulse generation is not simply a GH cell network response, but is shaped by a tissue microenvironment context, involving a functional association between the GIH cell network activity and fluid microcirculation. These new cellular in vivo imaging approaches will allow the future investigation of how the pituitary microenvironment influences different cell systems, not only during periods of normal physiological demand, but also when the endocrine tissue and microvasculature are altered (e.g. tumours).

AP1.2
Dynamic imaging of the tumor microenvironment: impact on invasion and CTL effector function
Peter Friedl1,2, Stephanie Alexander2 & Bettina Weigelin1
1Department of Cell Biology, Nijmegen Center for Molecular Life Science, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands;
2Rudolf-Virchow Center for Experimental Biomedicine and Department of Dermatology, Venerology, and Allergology, University of Würzburg, Würzburg, Germany.

Improvements in real-time intravital imaging techniques and two-photon microscopy make it now possible to visualize the complex tumor microenvironment in vivo, over time and deeply within intact tissues. Using dynamic imaging, we addressed central aspects of tumor progression, including dynamic interactions of invading tumor cells with the tumor microenvironment and the local regulation of tumor infiltrating immune cells in eradicating tumor cells. Immunological control of tumor progression requires the activation and expansion of tumor-specific cytotoxic T lymphocytes (CTL) followed by an efficient effector phase in the tumor lesion. To mimic in vivo dynamics of CTL effector function we established a 3D collagen matrix based assay to observe CTL effector function in real time, including active migration, sequential interactions with and serial killing of target cells. Individual CTL-target cell contacts were variable in duration (min to hours) and kinetics (stable to dynamic), comprised a median lag-phase of 90 min until apoptosis of the target cell, and were followed by CTL detachment and sequential killing of multiple target cells (up to 11/24 h).

Using this model, local factors present in the tumor microenvironment were identified that interfere with or enhance the anti-tumor CTL response and serial killing. CXCL12, a chemokine with pro-migratory effects on CTL and known to be upregulated in most tumors, enhanced CTL migration, shortened interaction times with target cells and reduced the killing efficiency on a per-contact-basis which lead to near-complete abrogation of target cell killing at low CTL to target cell ratio. This effect was reversed by a CXCR4 antagonist, suggesting that enhancing serial killing by targeting the CXCR4/CXCL12 axis may improve anti-tumor immunotherapy.

Pro-migratory signals mediated by the tumor microenvironment further contribute to cancer invasion and metastasis. We investigated in vivo tumor invasion principles, potential guidance structures, the molecular mechanisms of invasion, and the response of different tumor niches to anti-cancer therapy. Intravital fluorescence and multiphoton microscopy was used to study HT-1080 fibrosarcoma xenografts implanted into the dorsal skin-fold chamber on nude mice. After initial growth, tumors developed zones of invasive growth consisting of multicellular collective invasion strands that retained cell-cell contact and the ability to proliferate while invading at velocities of up to 200 μm/day. This invasion occurred in a directed manner along blood/lymph vessels and muscle strands of the deep dermis. Therapeutic irradiation induced complete regression of the tumor main mass yet failed to eradicate perivascular invasion strands. Using knockdown and/or antibody-based treatment against β1/β3 integrins but not interference with EGFR prompted significant radiosensitization of both, tumor main mass as well as invasion strands implicating integrin-mediated adhesion and/or anti-apoptotic signaling (anoikis) in radioreistance. In conclusion, collective invasion not only supports fast angiotropic invasion but maintains an integrin-dependent survival niche.

Thus, visualizing cell kinetics in 3D in vitro and in vivo models provides novel cellular and molecular mechanisms of CTL-mediated immune defense and cancer invasion, and the role of the local tumor microenvironment therein.

AP1.3
Quantitative imaging for assessing physical phenotypes in stem cells
Kevin Chaitl, Andrew Ekpenyong & Jochen Guck
University of Cambridge, Cambridge, UK.

It is becoming increasingly clear that stem cell function and differentiation state are affected by the physical environment of the stem cell, and the stem cell’s physical properties – or physical phenotypes. Physical phenotypes include how the cell responds to forces in its environment and its subcellular structure. This presentation will be primarily focused on subcellular structure, or structural phenotype. I will present two different techniques to quantitatively assess structural phenotype. The first is digital holographic microscopy (DHM), which is a marker-free method for assessing protein density within cells. The second is confocal microscopy, in which I used an anti-HIP1a label for heterochromatin to deduce the distribution of chromatin and how it changes during cell differentiation. With both of these techniques, I observed significant changes in the structural phenotype of HL60 cells as they differentiated into three different lineages. I extended these techniques to pluripotent stem cells (PSCs), and assessed how the subcellular structure changed as PSCs make their first fate decisions. I will discuss the changes observed in the distribution of chromatin, novel ways to quantify it, and how it can be used as a biomarker for stem cell function and differentiation state. Furthermore, I will discuss how physics-based concepts and techniques can lead to a minimally invasive, quantitative toolbox for assessing stem cells, both in the laboratory and in the clinic.
Clinical Guidelines
Pituitary apoplexy new society guidelines for treatment
John Wass, Senthil Kumar, Narendra Reddy, Mark Vanderpump & Stephanie Baldeweg
Churchill Hospital, ORH, Oxford, UK.

The guidelines for the treatment of pituitary apoplexy have now been published in Clinical Endocrinology (Clin Endocrinol 2011 Jan;74(1):9–20). These resulted from a group set up during the London pituitary multidisciplinary meeting. Classical pituitary apoplexy is a medical emergency and rapid replacement with hydrocortisone maybe life saving. It is a clinical syndrome characterised by the sudden onset of headache, vomiting, visual impairment and decreased consciousness caused by haemorrhage and/or infarction of the pituitary gland. It is associated with the sudden onset of headache accompanied or not by neurological symptoms involving the second, third, fourth and sixth cranial nerves. If diagnosed patients should be referred to a multidisciplinary team comprising, amongst others, of a neurosurgeon and an endocrinologist. Apart from patients with worsening neurological symptoms in whom surgery is indicated it is unclear currently for the majority of patients whether conservative or surgical management carries the best outcome.

Post apoplexy
There needs to be careful monitoring for recurrence of tumour growth. It is suggested that further trials be carried out into the management of pituitary apoplexy in order to optimise treatment.
Special Interest Groups
Bone and mineral special interest group

SIG1.1
Osteoporosis and other causes of high bone mass
Celia Gregson
University of Bristol, Bristol, UK.

Knowledge of rare genetic skeletal disorders has helped guide innovative treatment developments for osteoporosis, for example from our understanding of pyedysostosis balicaica has emerged, as have anti-SOST antibodies from our experience of sclerosteosis and Van Buchem’s disease. Yet much high bone mass remains unexplained, better appreciation of which may translate into improved understanding of bone regulation and new therapeutic targets for future osteoporosis therapies.

This presentation will review disorders of osteoclast function known to cause osteoporosis in adults, in addition to disorders of enhanced bone formation leading to increased bone mass, for example those caused by mutations affecting LRP5 and SOST, which upregulate Wnt signalling. I will then describe the epidemiology of the high bone mass phenotype in the UK, which to date has not been explained by known mutations in LRP5 or SOST. I will also summarize some established diagnoses, aside from high bone mass, which can cause raised bone density on routine DXA scanning.

Finally, I will present details of the clinical features of unexplained high bone mass, which include increased skeletal and mandibular size and impaired buoyancy, suggestive of a mild skeletal dysplasia, although the underlying genetic basis remains to be determined. Interestingly, these high bone mass cases also have evidence of a metabolic phenotype as evidenced by an increase in fat mass, providing support for recent laboratory studies suggesting that important links exist between bone and fat metabolism.

SIG1.2
Patients teaching doctors: hypophosphatemic rickets and the revelation of a novel phosphate homeostatic system
Thomas Carpenter
Yale University, New Haven, Connecticut, USA.

Familial hypophosphatemic rickets was recognized in the 1950s, when hypophosphatemia due to renal phosphate wasting was identified in individuals with rickets unresponsive to vitamin D. X-linked dominant inheritance was evident in many cases, and the most common form of the disease is known as X-linked hypophosphatemia (XLH). A description of vitamin D-refractory rickets likely represents the first report of XLH (Albright F et al, Am J Dis Children 1937). After trials of phosphate therapy, and then with vitamin D, combination therapy using calcitriol together with phosphate salts eventually emerged as the standard of care for XLH in the 1980s. This therapy usually improves, but does not completely heal, rachitic deformities and short stature. Later complications include eventual development of osteophytes, paradoxical calcification of tendons and ligaments, and osteoarthrits, all of which are poorly understood. The mutated gene in XLH, PHEx, is a product of the osteocyte, but its role in the pathogenesis of phosphate wasting is poorly understood.

Study of XLH and its related disorders has led to the identification of a novel fibroblast growth factor family member, FGF23, a unique FGF with endocrine properties. Renal action of FGF23 leads to reduced expression of the type II sodium-phosphate co-transporters NaPi-IIa and NaPi-IIc which are instrumental in renal tubular phosphate reclamation, as well as to reduced expression of CYP27B1, which encodes the vitamin D 1-hydroxylase enzyme. Selectivity of the renal tubular action of FGF23 is mediated by a transmembrane protein, klotho, an essential co-receptor for FGF23, converting generic FGF receptors to specific FGFR5 receptors. The osteocyte is the primary source of FGF23; thus this novel phosphate regulatory system serves as a mechanism by which the mineralizing skeleton can communicate mineral abundance or demand to the kidney and thereby signal the excretion or conservation of this important component of the skeleton.

SIG1.3
Osteogenesis imperfecta
Mark Cooper
College of Medical and Dental Science, University of Birmingham, Birmingham, UK.

Osteogenesis imperfecta (OI) is a rare but serious genetic disorder of bone leading to increased fragility and greatly increased susceptibility to fracture. Some patients experience additional problems such as abnormally formed teeth, progressive deafness and scoliosis. The condition is divided into various subtypes with types I and IV being the commonest, type 2 is lethal in utero and type 3 is associated with most disability and deformity. Patients with OI typically have a large number of fractures in childhood, a reduced number in early adulthood but there appears to be an exaggerated risk of fracture with increasing age. In the past, there was no treatment but children with the condition are now frequently treated with courses of i.v. bisphosphonates that increase the density of bone and improve mobility. It is far less clear how adult patients with OI should be managed. This is largely due to a lack of long-term data regarding the natural history of untreated OI. Additionally, there is some evidence in support of the use of bisphosphonates in adults with OI but it is unclear who these drugs should be targeted to (and when) and whether there are any long-term adverse effects associated with these drugs in such patients. Long-term use of bisphosphonates has been linked to atypical fractures, suppressed bone turnover and osteonecrosis of the jaw in patients with osteoporosis. Whether similar risks exist in patients with OI is unclear. To address these issues we recently developed a combined bone-clinical genetics clinic within the West Midlands. We have identified over 200 individuals with OI, the majority of which have not previously had specialist assessment as adults. This cohort will provide the basis for longitudinal observational studies relating to the natural history of treated and untreated OI.

SIG1.4
Osteoporosis
Andrè Uitterlinden
The Netherlands.

Abstract unavailable.

Obesity special interest group

SIG2.1
Gut hormone based polypharmacy: same benefits without the surgery?
D Perez-Tilve
Division of Endocrinology, Departments of Medicine and Psychiatry, Metabolic Diseases Institute, University of Cincinnati College of Medicine, Cincinnati, Ohio, USA.

Obesity is reaching epidemic levels especially in western countries and has become a major public health issue because it is frequently associated with other comorbidities such as hypertension, dyslipidemia and diabetes. Therefore, there is increasing interest in the development of therapies against obesity that can offer additional improvements in those associated comorbidities. Unfortunately the current pharmacological treatments have demonstrated limited efficacy, leaving bariatric surgery as the only treatment that promotes a substantial reduction of body weight and in glycemic control. With the aim of establishing new pharmacological alternatives to the use of bariatric surgery, we have developed new single molecules able to activate several hormone receptors involved in the control of energy balance. We have previously proved the efficacy of a peptide with coagonist activity for the glucagon-like peptide-1 (GLP-1) and glucagon receptor, on body weight loss and glycemic control in rats and mice. Now we have designed a novel single peptide coagonist that activates both GLP-1 and glucose-dependent insulinotropic peptide (GIP) receptor. This coagonist is protected from degradation in plasma by dipeptidyl peptidase IV (DPP-IV) and shows a long-lasting activity in rodent models, inducing a greater reduction in body weight and improving glycemic control than other peptides with single agonist activity for the GLP-1 receptor, currently available therapies for the treatment of type 2 diabetes. Our data suggest that the rational design of peptides that can simultaneously modulate the activity of several receptors involved in the control of energy metabolism offers new pharmacological alternatives for the treatment of obesity and diabetes.

SIG2.2
Gut hormones, weight loss and metabolic improvement after bariatric surgery
Torsten Olbers
Imperial College, London, UK.

Background
Bariatric surgery is currently the only evidence based treatment for morbid obesity. However, we have limited knowledge regarding the mechanism of action in many of procedures used.
Method

This is a review of the results from studies on weight loss, metabolic normalisation and mechanism of action in patients undergoing bariatric surgery.

Results

In the Swedish Obese Subject (SOS) study 2000 patients who underwent bariatric surgery was compared to a conservatively treated matched control group (n = 2000). Surgical mortality was 0.25%. The outcome revealed a significant and sustained weight loss of in mean 16% over 10–15 years in the whole group (25% after gastric bypass). Furthermore the QoL, was improved in operated group as were most cardiovascular risk factors. We also found an adjusted reduction in mortality of 30% in the surgical group and a 40% reduction in cancer incidence in women. Gut hormone release after gastric bypass demonstrates a ‘supraphysiological’ release of GLP-1, PYY and other gut hormones promoting satiety after test meal, but no increase in fasting state. These effects along with changes in GIP, glucagon, etc. might contribute to weight independent improvements in type 2 diabetes after gastric bypass surgery.

Conclusion

Bariatric surgery, and especially gastric bypass, is associated with substantial and sustained long-term weight loss. This leads to a normalisation of many metabolic risk factors and importantly also reduction in overall mortality and incidence of cancer. The mechanism of action in gastric bypass surgery appears to be complex and includes altered gut hormonal response to a meal.

Laboratory aspects of clinical endocrinology special interest group

SIG2.3

Pre- and post-operative management of patients undergoing bariatric surgery

R Andrews
Department of Exercise, Nutrition and Health Science, University of Bristol, Bristol, UK.

Current evidence suggests that bariatric surgery offers the best hope for substantial and sustainable weight loss in patients with morbid obesity. These facts, coupled with the improved minimally invasive procedures, have driven a four-fold increase in the number of bariatric operations performed over recent years in the UK.

Bariatric surgery though is not suitable for everyone. A BMI > 50, Age > 50, smoking, sleep apnoea and CVD disease all increase the risk of death and complications. Weight loss is often suboptimal in patients who have significant psychiatric disease, those who are less well informed, or not motivated.

Pre-operative evaluation and education of patients ensures that expected benefits of the operation outweigh the risks. This involves determining a patient’s indication for surgery, identifying issues which may interfere with the success of surgery, and assessing and treating co-morbid diseases. Typically assessment includes psychological testing, nutrition evaluation and medical assessment.

Successful weight loss and prevention of complications post operatively require close follow-up by a multidisciplinary team. Frequent blood tests and nutritional input is needed to prevent micronutrient deficiencies and maintain good eating behaviour. For gastric bands regular fills are needed to reduce satiety but not cause undue restriction. Medical input is also needed to prevent weight regain and manage the co-morbid diseases.

This talk will provide clear guidance on how to assess, prepare and manage patients undergoing bariatric surgery.

SIG2.4

Bariatric surgery: a cure for diabetes?

D Johnston
Barking, Havering and Redbridge University Hospitals NHS Trust, London, UK.

Type 2 diabetes is increasing in prevalence and is associated with, amongst others, premature CVD, retinopathy and kidney failure. The aim of care is to minimise the complications through euglycaemia but in the long term, this is rarely possible. Treatment of hypertension and dyslipidaemia also has benefit and patients are often consigned to a lifetime of polypharmacy. Quality of life is affected, especially in those with complications and in those who need treatment with insulin. Therapy with some second line drugs (sulphonylureas, thiazolidinediones and insulin) often results in weight gain. Novel treatment modalities are being sought and newer agents, such as thiazolidinediones, GLP-1 receptor antagonists and DPP-4 inhibitors, exploit mechanisms of action different to those of older drugs. Bariatric surgery is increasingly performed and Roux-en-Y Gastric Bypass (RYGB) is the surgical procedure for which the evidence base is greatest, with reported diabetes ‘remission’ rates of > 80%. Most evidence derives from people who also had morbid obesity but recently, patients with lower BMIs have been studied. Irrespective of initial body weight, RYGB is followed by a fall in insulin resistance, detectable within days of surgery and thus unlikely to be caused simply by weight loss. There is also an improvement in insulin secretion including restoration of the first phase loss of which had previously been considered irreversible. Multiple questions remain, however, before advocating RYGB generally in diabetes management (in the absence of morbid obesity). Principal amongst these is the lack of randomised controlled trials comparing surgery with modern intensive medical management. RYGB is essentially irreversible and long term data are scanty. We do not know the subgroups of patients who will benefit most and management strategies for care following operation have not been defined. Comparisons of life quality and economic data are also scanty. While promising, much remains to be learned.

SIG3.2

Definition of male hypogonadism

Christina Wang & Ronald Swerdloff
Harbor-UCLA Medical Center and Los Angeles Biomedical Research Institute, Torrance, California 90509, USA.

The Endocrine Society guidelines define male hypogonadism as a ‘clinical syndrome’ where the diagnosis is based on symptoms or signs and unequivocally low serum testosterone (T) levels. Generally accepted by most clinicians, diagnosis of male hypogonadism is clinically relevant because T replacement can restore libido and correct sexual dysfunction, increase lean and decrease fat mass, increase in bone mineral density and vitality. Low serum T is associated with increased all cause mortality. Low serum T levels are commonly associated with aging, obesity, metabolic syndrome, type 2 diabetes and other chronic illness. Because symptoms of T deficiency are non-specific and variable, the diagnosis of male hypogonadism requires serum T concentration measurements. Serum T shows diurnal variation, and samples for T measurements should be drawn in the morning because comparison is made with reference ranges derived from morning samples of adult men. Methods to determine serum T including immunoassays based assays and platforms and liquid chromatography tandem mass spectrometry. The latter is regarded as the gold standard for serum T measurement. For the diagnosis of male hypogonadism all these methods are generally adequate. There are wide between laboratory and method differences.

The Endocrine Society guidelines define male hypogonadism as a ‘clinical syndrome’ where the diagnosis is based on symptoms or signs and unequivocally low serum testosterone (T) levels. Generally accepted by most clinicians, diagnosis of male hypogonadism is clinically relevant because T replacement can restore libido and correct sexual dysfunction, increase lean and decrease fat mass, increase in bone mineral density and vitality. Low serum T is associated with increased all cause mortality. Low serum T levels are commonly associated with aging, obesity, metabolic syndrome, type 2 diabetes and other chronic illness. Because symptoms of T deficiency are non-specific and variable, the diagnosis of male hypogonadism requires serum T concentration measurements. Serum T shows diurnal variation, and samples for T measurements should be drawn in the morning because comparison is made with reference ranges derived from morning samples of adult men. Methods to determine serum T including immunoassays based assays and platforms and liquid chromatography tandem mass spectrometry. The latter is regarded as the gold standard for serum T measurement. For the diagnosis of male hypogonadism all these methods are generally adequate. There are wide between laboratory and method differences.

The Endocrine Society guidelines define male hypogonadism as a ‘clinical syndrome’ where the diagnosis is based on symptoms or signs and unequivocally low serum testosterone (T) levels. Generally accepted by most clinicians, diagnosis of male hypogonadism is clinically relevant because T replacement can restore libido and correct sexual dysfunction, increase lean and decrease fat mass, increase in bone mineral density and vitality. Low serum T is associated with increased all cause mortality. Low serum T levels are commonly associated with aging, obesity, metabolic syndrome, type 2 diabetes and other chronic illness. Because symptoms of T deficiency are non-specific and variable, the diagnosis of male hypogonadism requires serum T concentration measurements. Serum T shows diurnal variation, and samples for T measurements should be drawn in the morning because comparison is made with reference ranges derived from morning samples of adult men. Methods to determine serum T including immunoassays based assays and platforms and liquid chromatography tandem mass spectrometry. The latter is regarded as the gold standard for serum T measurement. For the diagnosis of male hypogonadism all these methods are generally adequate. There are wide between laboratory and method differences.

The Endocrine Society guidelines define male hypogonadism as a ‘clinical syndrome’ where the diagnosis is based on symptoms or signs and unequivocally low serum testosterone (T) levels. Generally accepted by most clinicians, diagnosis of male hypogonadism is clinically relevant because T replacement can restore libido and correct sexual dysfunction, increase lean and decrease fat mass, increase in bone mineral density and vitality. Low serum T is associated with increased all cause mortality. Low serum T levels are commonly associated with aging, obesity, metabolic syndrome, type 2 diabetes and other chronic illness. Because symptoms of T deficiency are non-specific and variable, the diagnosis of male hypogonadism requires serum T concentration measurements. Serum T shows diurnal variation, and samples for T measurements should be drawn in the morning because comparison is made with reference ranges derived from morning samples of adult men. Methods to determine serum T including immunoassays based assays and platforms and liquid chromatography tandem mass spectrometry. The latter is regarded as the gold standard for serum T measurement. For the diagnosis of male hypogonadism all these methods are generally adequate. There are wide between laboratory and method differences.
Hypothyroidism has been defined as those conditions which result in suboptimal circulating levels of thyroid hormones. The problem is how to define such suboptimal levels? In the 1970s, the advent of routine assays for TSH together with the concept that the most sensitive ‘peripheral tissue’ to diminished circulating thyroid hormone concentrations is the pituitary led to the acceptance that elevated serum TSH was required to define primary hypothyroidism. There are problems, however, in defining what is an elevated serum TSH and what serum concentrations of TSH should trigger thyroxine replacement therapy. Routine TSH assays show considerable between method biases, which do not appear to correlate with the TSH reference ranges suggested by the manufacturers. These assay bias differences are often ignored in research communications that present data supporting clinical action limits for the implementation of T4 therapy. Furthermore it has been suggested that the log normal distribution of TSH observed in the ‘healthy’ population may arise through the inclusion of subjects with occult hypothyroidism. If this were the case then the upper reference limit for TSH should be significantly lower than that currently used by most laboratories. In this lecture these problems and current evidence will be discussed.

Polycystic ovary syndrome (PCOS) is a common disorder which has considerable phenotypic variability and this has led to controversy over its exact definition and diagnosis. Over the past years a number of definitions have been developed by professional associations. These consensus statements have used a combination of clinical, laboratory and imaging studies as the defining criteria. However, the statements imply that these measures are dichotomous variables without considering factors such as normal physiology, observer subjectivity or measurement variability on the outcome. Published data would suggest that there is considerable uncertainty of all the measurements and that there is a complete lack of clarity of the definition of the term ‘hyperandrogenaemia’ - and all the factors can lead to misdiagnosis. This paper proposes that the current diagnostic strategies for PCOS are defined too vaguely to be certain that individuals fit the definition of the syndrome. A pragmatic approach may be taken in the management of an individual depending upon her particular symptoms and needs. However, research into the epidemiology, pathophysiology and treatment of PCOS will require the production of robust definitions of the diagnostic criteria.
Meet the Expert Sessions
Generously supported by
Clinical Endocrinology
MTE1
Abnormal growth and puberty presenting in late adolescence
Peter Clayton
Manchester Academic Health Sciences Centre, Manchester, UK.

Young people in their late teenage years may present to either paediatric or adult endocrine clinics with abnormal growth (usually as short stature and/or recognition of slowing growth) and delayed or absent puberty. Anxieties about growth potential may be very significant, and the lack of puberty may blight social interactions and markedly reduce self-esteem.

Although constitutional delay in growth and puberty is the most common diagnosis for this type of presentation, there are a number of other significant aetiologies which must be considered. These include – chromosome disorders, e.g. 47XXY, hitherto undiagnosed genetic disorders, e.g. hypogonadotropic hypogonadism including Kallmann’s syndrome, and acquired lesions in the hypothalamic-pituitary axis, such as craniopharyngioma.

It is therefore essential that there is close attention paid to family history of related disorders, social and educational history, co-morbidities (e.g. sense of smell), symptoms and signs of endocrinopathy (including diabetes insipidus) and CNS signs.

Investigations may be minimal (e.g. TSH, bone age) if there is a clear family history of delayed puberty and clinical examination reveals that puberty has commenced. However investigations may need to be extensive including assessment of pituitary function (both basal and dynamic testing) and brain imaging.

Treatment should be directed at the underlying disorder. There are many regimens for pubertal induction, but starting doses of sex steroids should be tailored to the degree of pubertal development already achieved and the need to promote growth – low doses for those with absent or minimal pubertal signs (e.g. testosterone injections 50 mg monthly or ethinylestradiol orally 5 µg daily), and higher doses for those already established into puberty or with pubertal arrest.

This clinical scenario is an excellent example of where both paediatric and adult endocrinologists need the expertise to manage these young people, and seeing them within a joint clinic is a good strategy.

MTE2
Pitfalls in diagnosis and treatment of Cushing’s syndrome
Xavier Bertagna
Cochin Hospital, Paris, France.

Many difficulties can be encountered in the management of patients with Cushing’s syndrome or suspected Cushing’s syndrome.

In the first step of the diagnosis strategy, establishing the state of chronic hypercortisolism may be hampered by a number of pitfalls: drug interaction (inducers of high CBG levels, liver enzyme inducers, glucocorticoids, antiglucocorticoid RU 486, Glycericytolic acid); intercurrent pathologic states (obesity, thyroid dysfunction, renal failure). Various pathologic or physiologic conditions may be associated with biochemical, and sometimes clinical, evidences of endogenous glucocorticoid excess creating the ‘pseudo-Cushing’ syndrome (depression, anorexia nervosa, alcoholism, strenuous exercise, pregnancy).

Familial resistance to glucocorticoids, a rare inherited condition, is associated with excess glucocorticoid, androgen, and mineralocorticoid production with no clinical manifestations of Cushing’s syndrome. Normal suppression with the classic low-dose dexamethasone test can be observed in authentic Cushing’s disease.

Once the positive diagnosis of Cushing’s syndrome has been convincingly established, one may deal with numerous etiologic pitfalls. Cushing’s disease mimicking an autonomous adrenocortical tumor, severe Cushing’s disease mimicking the classic ectopic ACTH syndrome, mild ectopic ACTH syndrome mimicking the classic Cushing’s disease. These various situations, and the ways to avoid pitfalls, will be presented and discussed using illustrating clinical cases.

MTE3
The difficult Cushing’s or Nelson’s patient
Peter Clayton
Manchester, UK.

Abstract unavailable.

MTE4
Managing Turner Syndrome through childhood and adolescence
Malcolm Donaldson
Glasgow University, Glasgow, UK.

Turner syndrome (TS), defined as loss or abnormality of the second X chromosome in a phenotypic female, affects 1 in every 2500 live female births. Around 155 females will be born with TS in the UK each year, with ~2800 girls ≤18 years and ~6500 women aged 18–60 years living with the condition.

Short stature is a constant feature with gonadal dysgenesis present in ~90%. Associated features include dysmorphic features which are often mild, lymphoedema, otitis media with effusion, sometimes followed by suppurative otitis media ± cholesteatoma; bicuspid aortic valve, coarctation and dilatation of aortic root; hypertension; and an autoimmune diathesis. IQ is usually normal but there may be specific learning difficulties (commonly with mathematics) and a degree of social vulnerability.

Optimal management of TS begins with timely diagnosis followed by thorough counselling and education of the family. Growth hormone (GH), of proven value in improving final height, is usually started aged 5 years (earlier if the girl is especially short) in the dose of 10 mg/m2 per week given as daily s.c. injections.

In the UK pubertal induction is currently with a 3-yr low dose ethinyl oestradiol protocol, usually started at 12–13 years. Four-monthly outpatient review is required to monitor growth and development and for the surveillance of blood pressure, middle ear disease (present in over 60%), educational difficulties (often compounded by conductive hearing loss), and any psychosocial or family problems (which affect compliance with GH). Bone age, thyroid function and IGFI are checked annually; gonadotrophins, pelvic/renal and cardiac ultrasound at diagnosis, 11–12 years (prior to pubertal induction) and at adult transfer.

At 16–18 years joint review by the paediatric endocrine team with an adult colleague – gynaecologist, endocrine or reproductive physician – ensures smooth transition. Adult care involves lifelong surveillance of cardiovascular, reproductive, otological, endocrine and metabolic bone health.

MTE5
Late effects of cancer therapy
Richard Ross
University of Sheffield, S Yorks, UK.

One in eight hundred young adults is now a survivor of childhood cancer as a result of tremendous advances in cancer therapy. However, this success now brings with it the challenge that both the cancer and its therapy may have late effects. In a recent review of 10 397 young adult survivors, 62.3% had at least one chronic condition; 27.5% had a severe or life-threatening condition (grade 3 or 4). The adjusted relative risk of a chronic condition in a survivor, as compared with siblings, was 3.3 (95% CI, 3.0 to 3.5); for a severe or life-threatening condition, the risk was 8.2 (95% CI, 6.9 to 9.7). Among survivors, the cumulative incidence of a chronic health condition reached 73.4% (95% CI, 69.0 to 77.9) 30 years after the cancer diagnosis, with a cumulative incidence of 42.4% (95% CI, 33.7 to 51.2) for severe, disabling, or life-threatening conditions or death. Owing to a chronic condition. Thus, late effects are common and many of these late effects are endocrine in nature including; hypogonadism, infertility and hypopituitarism. This expert session will address the management of endocrine late effects following cancer therapy.

MTE6
Assessing fracture risk and response to therapy in osteoporosis
Eugene McCloskey
University of Sheffield, Sheffield, UK.

Osteoporosis is a skeletal disorder characterized by compromised bone strength predisposing to an increased risk of fracture. An individual with a hip or vertebral fracture has an excess risk of death that is highest during the first year. Moreover, all osteoporosis related fractures can lead to significant long-term disability and decreased quality of life. The ability to accurately gauge fracture risk is critical in identifying cost-effective thresholds for intervention.

In 2008, the WHO Collaborating Centre for Metabolic Bone Diseases at Sheffield released the fracture risk assessment tool (FRAX) for estimation of individualized 10-year probability of hip and/or vertebral fracture (composite of hip, clinical spine, distal forearm, and proximal humerus) with or without BMD. The FRAX tool integrates seven clinical risk factors (prior fragility fracture, a parental history of fracture, female sex, age, body mass index, smoking status, and use of hormone replacement therapy).
of hip fracture, smoking, use of systemic glucocorticoids, excess alcohol intake, body mass index (BMI), rheumatoid arthritis and other secondary causes of osteoporosis which, in addition to age and sex, contribute to a 10-year fracture risk estimate independently of BMD.

The importance of this tool in clinical practice is highlighted by the fact that many recently published clinical guidelines recommend pharmacological treatment on the basis of 10-year fracture risk. A key question that arises is the reversible nature of the risk that is identified. Several studies now demonstrate that patients at high probability of fracture have underlying low bone mass and respond to anti-osteoporotic therapies.

The current platform at the WTCHG, Illumina HiSeq2000, is capable of supporting a wide variety of applications that are changing research. Applications range from whole genome to all exon re-sequencing for studies of complex disease traits, allowing variants to be detected. The world of expression analysis is now becoming cost effective through sequencing. Not only is RNA-Seq more sensitive than microarray for relative gene expression, its dynamic range is larger, allowing identification of allele specific expression, splice variants, SNPs and regulatory RNA’s. Coupled with this, applications have been developed to support genome-wide epigenetic analysis.

With researchers rapidly developing new protocols to allow them to make use of High Throughput Sequencing for their own research, this is just the beginning …
Nurse Session
Turner/Klinefelter’s/Noonan syndrome: case presentations

N1.1
Turners/Klinefelters/Noonans syndrome
G Conway
London, UK.

Abstract unavailable.

N1.2
Klinefelter’s case presentation
P Pickett
Shrewsbury, UK.

Abstract unavailable.

N1.3
Living with TS: an adult viewpoint
H Cleaver
Turner Syndrome Support Society, UK.

Abstract unavailable.

N1.4
Klinefelter’s Syndrome Association
S Cook
Klinefelter’s Syndrome Association, UK.

Abstract unavailable.

Hyperthyroidism: case presentations

N2.1
Hyperthyroidism – overview of causes and treatment options
Jayne Franklyn
University of Birmingham, Birmingham, UK.

Hyperthyroidism is common, affecting up to 3% of the population. The major causes in the UK are autoimmune (Graves’ hyperthyroidism) and toxic nodular goitre. Despite the prevalence of hyperthyroidism, there is relatively little agreement regarding the relative roles of the three principle treatment options – antithyroid drugs, radiodine and surgery. The presentation will address these roles and specifically will address areas of controversy regarding treatment through the use of clinical scenarios. These will include the young female patient with Graves’ hyperthyroidism – pros and cons of thionamide therapy. The next scenario is the elderly male with known cardiovascular disease and AF – how best to use radioidine. The 3rd scenario will address the role of radioidine in the patient with ophthalmopathy, the 4th a patient with hyperthyroidism in early pregnancy, and finally the teenage patient with Graves’ hyperthyroidism. These scenarios will be used to assimilate evidence regarding best practice in these relatively common clinical situations.

N2.2
Nurse-led clinic and hyperthyroidism
Violet Fazal-Sanderson
Department of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK.

Background of NLC’s: The emergence of Nurse-led clinics (NLC) has given nurses a pathway for innovation and excellence towards providing safe, efficient, cost-effective and quality healthcare service.

Reasons for setting up a Thyroid NLC in Oxford: Patients were waiting up to 3 months before seen by a consultant. Funding was available for a part-time endocrine nurse.

Aims of the Thyroid NLC: To set up and provide a safe, efficient, cost-effective service for patients with uncomplicated hyperthyroidism (Graves’ disease, nodular goitre and thyroiditis). To reduce patient waiting time and to audit the service provided.

Components for setting up the NLC: Approval was obtained from the ORH NHS Trust. Appropriate knowledge and skills on the subject were acquired by the nurse. A clinic consultation room for the NLC and an endocrine consultant was made available to work along side. A clinic code was given for the purpose of documenting and monitoring patients attendances. Proformas were prepared for history taking, physical examination, monitoring/evaluating results and patient/GP correspondence. Slots were allocated to see 2 new and 4 follow-up patients per week and a direct access support-line was provided for patients.

Outcomes: Clinic has been running since 2005. Nurse prescribing course has enabled medications to be prescribed and drug dose adjustments to be made by the nurse. Between 9/09-9/10, there were 81 new and 185 follow-up attendances. The income generated during this time was £34,316. Non-face to face communications also generated income of around £3,000 per month. Two audits concluded the benefits of the NLC.

Challenges & future developments: To maintain professional competence and expertise of the nurse to run an efficient and high quality clinic through regular auditing.

To expand the service by setting up a Nurse-led post-radioactive iodine clinic.

N2.3
Hyperthyroidism – Case presentation
Dianne Wright
Specialist Nurse in Endocrinology, Bradford NHS Teaching Hospitals Foundation Trust, Bradford, UK.

This 32 year old lady was referred in 2008 with Graves Disease. She presented with typical symptoms which included tiredness, shaking, palpitations, itching, eye redness, ophthalmopathy, and exophthalmos. She had lost 3 stones over 3 months at Weight watchers. The GP had commenced carbimazole but she did not know the dose. In September 2008 results showed FT4 73.6, TT3 > 12.5, TSH < 0.05 and +TPO antibodies – > 1300. This lady declined a referral to the ophthalmologists as her eyes were not much of a concern for her. She failed to attend her next 2 appointments so was discharged back to her GP.

In October 2009 this lady was re-referred to the endocrine department. She failed to attend this appointment due to family problems and bereavements. She had stopped taking carbimazole due to mouth ulcers and boils. Clinically she was extremely hyperthyroid, with a moderate to large sized goitre and worsening of her eye condition. She had bilateral exopthalmos, reduced vision in the left eye, pain, grittiness, lid lag and lid retraction but she thought this was normal for her. Carbimazole and beta-blockers were re-commenced and she was referred to the ophthalmology department. She did not attend her next endocrine appointment and postponed her following 2 appointments.

In March 2010 a letter was sent to the patient requesting blood tests and her medication dose. In June 2010 she attended clinic saying she had taken carbimazole 20 mg BD for the last 2 weeks. She had walked out of the ophthalmology waiting area due to clinic delay. She had severe proptosis, double vision, aching eyes, inability to close her eyes at night but she did like the weight that she had lost. She also had a choking sensation with her goitre. At this point her medication was increased, thyroid function requested along with a thyroid ultrasound. She was re-referred to the ophthalmology department and the thyroid surgeon potentially for a thyroidectomy. In clinic in September 2010 she said she felt ‘horrible all the time’ but had missed her ultrasound, ophthalmology and thyroid surgeon appointments due to being away for 3 months before seen by a consultant. Funding was available for a part-time endocrine nurse.

Aims of the Thyroid NLC: To set up and provide a safe, efficient, cost-effective service for patients with uncomplicated hyperthyroidism (Graves’ disease, nodular goitre and thyroiditis). To reduce patient waiting time and to audit the service provided.

Components for setting up the NLC: Approval was obtained from the ORH NHS Trust. Appropriate knowledge and skills on the subject were acquired by the nurse. A clinic consultation room for the NLC and an endocrine consultant was made available to work along side. A clinic code was given for the purpose of documenting and monitoring patients attendances. Proformas were prepared for history taking, physical examination, monitoring/evaluating results and patient/GP correspondence. Slots were allocated to see 2 new and 4 follow-up patients per week and a direct access support-line was provided for patients.

Outcomes: Clinic has been running since 2005. Nurse prescribing course has enabled medications to be prescribed and drug dose adjustments to be made by the nurse. Between 9/09-9/10, there were 81 new and 185 follow-up attendances. The income generated during this time was £34,316. Non-face to face communications also generated income of around £3,000 per month. Two audits concluded the benefits of the NLC.

Challenges & future developments: To maintain professional competence and expertise of the nurse to run an efficient and high quality clinic through regular auditing.

To expand the service by setting up a Nurse-led post-radioactive iodine clinic.
Young Endocrinologists Session
Young endocrinologists’ prize lectures

YEP1.1
New insights into glucocorticoid receptor function
Laura Matthews
University of Manchester, Manchester, UK.

The current model of glucocorticoid receptor (GR) action is well established, whereby GR remains inactive in the cytoplasm until bound by ligand, then rapidly translocates to the nucleus to regulate target genes. However, our recent observations challenge the simplicity of this model and suggest a greater range of GR action.

We have identified a novel pathway in which the GR is recruited to the plasma membrane through binding to the lipid raft marker caveolin-1, and then couples to kinase cascades to mediate Gc effects. Using transcriptome profiling we found that many Gc-regulated genes require co-expression of caveolin-1 for their regulation, indicating that recruitment of GR to the plasma membrane is necessary to regulate a subset of Gc targets.

We have also identified a cytoplasmic role for the constitutive splice variant GRγ. The mechanism for ligand-independent gene regulation by GRγ appears indirect, through the activation of cytoplasmic kinases including JNK and subsequent recruitment of SPI1 and API transcription factors. GR therefore mediates some cellular effects in the unliganded state.

In further support of unliganded GR effects, we have identified cell-cycle driven phosphorylation which targets GR to the mitotic spindle, suggesting a role for GR in mitosis. Indeed, GR knockdown cells accumulate in metaphase, and show evidence of multiple spindle defects. It appears therefore, that during mitosis when cells are transcriptionally silenced, GR adopts a new ligand-independent role in directing accurate chromosome segregation.

We have therefore characterised a range of cellular effects that do not require nuclear translocation, and are influenced by GR isoform expression and ligand availability. We also reveal a novel role for GR as a regulator of mitotic progression. Identification of these novel pathways for GR function offer explanations for physiological phenomena including rapid steroid responses, and will inform novel approaches for selective anti-cancer drug development in inflammatory disease.

YEP1.2
Clinical, genetic and molecular characterisation of patients with familial isolated pituitary adenomas (FIPA)
H Chahal
Barts and The London School of Medicine and Dentistry, William Harvey Research Institute, London, UK.

There is increasing recognition that pituitary adenomas may occur in a familial setting, and a number of families have been identified to have familial isolated pituitary adenoma (FIPA), without features of the MEN1 syndrome, Carney complex, or other known familial disorders. Heterozygous germline mutations were identified in a gene encoding AIP (aryl-hydrocarbon receptor interacting protein) in some FIPA families. We have characterised our unique large collection of 140 families in terms of clinical presentation, age of onset, penetrance, genetic mutations, existence of other co-morbidities, responsiveness to somatostatin analogue therapy and histological characteristics.

FIPA is an autosomal dominant disease with a heterogeneous genetic background. A third of our families had a missense, nonsense, frameshift, splice-site, large deletion, and the first described promoter AIP mutation. We have identified a novel locus for another candidate gene involved in this condition; however, this remains to be fully identified.

In conclusion, detailed characterisation of FIPA patients, their mutations and the AIP pathway involved in AIP-related tumorigenesis together with the increasing recognition of a genetic background of familial and early-onset pituitary adenoma cases indicate that these findings are likely to have considerable significance to practising clinical endocrinologists.

A successful research career

YE1.1
Research in clinical academia
Miguel Debono
University of Sheffield, Sheffield, UK.

Why would you want to be a clinical academic? Why is research so enticing for some? Does intellectual stimulation overcome potentially lower salaries and longer hours of harder work? Yes, for some, the possibility of making a difference to medical knowledge is provoking and stimulating. Clinical academia gives researchers the possibility to express their own unique characteristics and traits including creativity and communication skills, professionalism and humanism, excitement, perseverance and mental strength.

There are a variety of ways to get into research. It may start off as early as medical school, where one through an intercalated bachelor of science (BSc), a medical science degree (BMedSci) or MB/PhD programme integrates a period of research within clinical education. This gives medical students the opportunity to demonstrate interest and experience in a science at a very early stage in their career and helps them discover whether they have the necessary aptitude for research. The next possible routes into academia are either through academic foundation programmes or through academic specialist training where one obtains a combination of clinical experience and exposure to an academic and research environment. This then provides academic clinical fellows the possibility of securing funding for a PhD or MD, provided by Universities or Biomedical Research Units/Centres, or even the option of applying for a competitive training fellowship, such as those provided by the National Institute of Health Research, the Medical Research Council or Wellcome trust. After

Endocrine Abstracts (2011) Vol 25
this stage, if committed to an academic pathway, one may apply for a clinical lectureship followed by a clinician scientist award or senior clinical lectureship. In addition, if not on an academic pathway, doctors may still decide to spend some time out of their speciality training and getting research experience; this potentially leading to a higher degree.

When applying for speciality training my intention was to pursue an academic career in Endocrinology, so when the opportunity arose in 2006 I jumped at the chance of obtaining a three year NIHR Academic Clinical Fellowship. I succeeded in attaining this post at the University of Sheffield. My job over the three years included a nine month research phase in the Department of Human Metabolism where I was exposed to research programmes focussing on diagnostic and management strategies of patients with Cushing’s and on the development of novel formulations of glucocorticoid replacement therapy. With an aim to concentrate my efforts on the effects of glucocorticoids and sex steroids on bone, I extended my fellowship to include a year working as an NIHR Academic Clinical Fellow in Bone Metabolism, in the Sheffield Bone Biomedical Research Unit and this has lead to me obtaining funding to perform a PhD. Full-time clinical research has proved to be challenging and exciting. Achieving targets is extremely satisfying and an academic career is highly recommended.

Managing your research career
A McNeilly
MRC Human Reproductive Sciences Unit, Edinburgh, UK.

In these times of restricted funding, it becomes crucially important that you position yourself to be the only possible candidate for future employment in a postdoctoral position or any other career path that you chose. The time to do this is during your PhD and MD studies and during your first postdoc. There are no hard and fast rules about how you do this, but some pointers may help. First, you must have a passion for research if this is what you want to do. If this is a job from 0900 to 1700 h monday to friday, then I suggest that you immediately start looking for alternative employment. Research is not like that and many breakthroughs have come from lateral thinking and for this you need to keep an open mind and be aware of opportunities. Thus you should go to all the seminars etc. that you can since you will either find it most boring or the most amazing talk that you have ever heard. However, often some gem of an idea will emerge, a link to the research you are doing, or a technique that you had not considered. In addition, in the face of the enormous deluge of publications in every field of research it is often difficult to keep up with advances merely by reading, and certainly by only reading abstracts. Thus by expanding your horizons you will become aware of other areas of research or techniques by osmosis. Then when faced with an interview for your next position you can say that you either have first hand knowledge of the methods, have worked beside someone who has used them, or that you are fully aware of the possibilities of such methods or techniques. Finally, a good mentor is very important.

Postdoctoral research – the things I wish I knew
Aylin Hanyaloglu
Imperial College London, London, UK.

Many PhD graduates will pursue a postdoctoral training position, most commonly in an academic laboratory. What can you expect from this period in your career? Do you need to go abroad to do a postdoc? What could/should you do to benefit the most of this time? And how can you use your time as a postdoc to help you with your career path once you leave?

I recall being very excited about beginning my postdoc and leaving the ‘PhD nest’. I trained and stayed an unanticipated five years at UCSF, San Francisco, before coming back to the UK as an independent researcher. In this session I will share my experiences during those five years, and that of fellow postdocs, from the time of pursuing these positions to the transition of postdoc to lecturership.

I will discuss the highs, lows and unexpected realities of this period of training that myself and others experienced. As a postdoc, you are in a unique position between not having to worry about completing a PhD, yet also without the role juggling and responsibilities of an independent academic. So to current PhD students thinking of postdoctoral research, or current postdocs, this period in your career will represent an exciting time with a tremendous amount of freedom to devote to your research and to explore possible career paths. With postdoctoral training periods getting longer and the unpredictable nature of research and the job market, there is an increased awareness of the need for long-term planning and to use this time to develop the necessary training in your postdoc. This should enable a successful move on to the next step of whatever chosen career path that is sought.

Commercialisation and IP – translating scientific research
Scott Webster
University of Edinburgh, Edinburgh, UK.

Commercialisation and the development of new technologies may be thought of as the natural conclusion of academic research. Typically this has been the preserve of industry, however, more recently translation has become a key aspect of any scientific research programme. The processes involved in the translation of basic and clinical research will be discussed in the context of an in-house drug discovery programme, highlighting the differences between hypothesis and goal-driven research. Funding streams, intellectual property and exit strategies will also be discussed.
Senior Endocrinologists
Session
The metamorphosis of testosterone from a sex steroid to a universal health factor
Eberhard Nieschlag
Centre for Reproductive Medicine and Andrology, University Hospitals Münster, Münster, Germany.

When in the 1930s testosterone was isolated, synthesized and introduced to the clinic, it was considered predominantly a sex steroid to be used for the treatment of erectile dysfunction and hypogonadism. In the 1950s the anabolic effect was ‘discovered’ and triggered the misuse of high doses of testosterone in sports, still prevailing today. Consequently, pharmacologic research concentrated its efforts on anabolic steroids, i.e. testosterone analogues hopefully without sexual effects, thus neglecting the search for badly needed improved forms of testosterone application. Improved modes of application only became the focus of the pharmaceutical industry when in the 1990s, the increasing life expectancy of males and the anticipated parallel increase in late-onset hypogonadism (LOH) precipitated the invention of transdermal testosterone preparations, finally producing the desired physiological testosterone levels. Intensified research on the aging male revealed the role of testosterone in the pathogenesis of obesity, diabetes type II and the metabolic syndrome as well as in osteoporosis, sarcopenia, atherosclerosis and coronary heart disease. Indeed, testosterone levels in blood are now considered a general predictor of morbidity and mortality. Thus, while the role of testosterone in erection and sexual function (supplemented by phosphodiesterase-5-inhibitors if needed) remained, its spectrum of action broadened to become a universal health factor.

Will we ever discover the mechanism of hormone action?
Jamshed Tata
MRC National Institute for Medical Research, London, UK.

Even before the identities and structures of many hormones were established, endocrinologists and physiologists had speculated about the mechanism of their action. A hundred years later, we are still struggling to establish in molecular and cellular terms the mode of physiological action of a given hormone. Two major reasons account for this situation: i) the failure to recognise that hormones as signalling molecules have been highly conserved during evolution but that their physiological actions vary enormously in different organisms or from one tissue to another in the same species; ii) that most investigations aiming to reveal the mechanism of action have been technology-driven and not hypothesis-based, as will be illustrated with a few time-lines. Despite these shortcomings, a major advance was made with the introduction of the concept of hormone receptors, which, thanks to the advent of gene cloning technology, are now molecularly and structurally well defined as key elements of hormone action. They are cellular homologues of the oncogenes c-erbA, located in the cell membrane or as transcription factors in the nucleus, respectively. Current thinking on understanding of how hormones act at the cellular and molecular levels is now focused on convergence of signalling pathways from different cellular locations, attempts to understand the immediate consequence of hormone-receptor interaction and the wider involvement of genomic and non-genomic networks. In view of what may sound as a rather negative account, one may well ask: Is it worth continuing to look for mechanisms of hormone action? The answer is most emphatically yes. So many concepts of cellular signalling mechanisms have emerged from work on hormone action. Just consider the discovery of cyclic AMP and the functions of several transcription factors.

An exploration of colour theory
Paul Belchetz
Leeds Nuffield Hospital, Leeds, UK.

Colour perception depends on light, photoreception and central processing in the nervous system. Newton, in 1666, showed the coloured spectrum formed from white light passed through a prism underwent no further splitting on passing through a second prism. Newton favoured the corpuscular theory of light, opposing the wave theory of Huygens, which was taken up about 1800 by Thomas Young to explain experiments on diffraction and later formulated the trichromatic theory of human colour perception, independently rediscovered by Helmholtz and supported by Maxwell’s work on electromagnetic radiation. Modern physics encapsulates both elements, post Einstein and Planck. Landmarks in understanding colour vision include description of retinal topography and physiology, especially elucidation of rod and cone function. Humans have three types of cones with peak light absorption at 450 nm (blue receptor), 530 nm (green), 560 nm (yellow). These properties depend on small variations in cone pigments, which single chains of about 350 amino acids containing seven transmembrane helices – the super family including many hormone receptors. Embedded in the pigment proteins of rods and cones is the same chromophore 11-cis-retinal, which on light absorption straightens the side chain, altering the protein shape, which in turn catalyses the downstream biochemical and electrical events signalling on to the bipolar cells and then the ganglion cells of the retina. These early stages encode topographically defined information about colour, contrast and provide substrates for phenomena such as after-image and shadow colours. The ganglion cells axons pass along the optic nerves, via the optic chiasm (cave putatory adomenas?) to the lateral geniculate bodies, whence further connections lead to the occipital cortex. Centrally there are less well understood phenomena: colour constancy and relativism discussed by Monge, and yellow–blue, red-green antagonistic systems postulated during the mid-19th-century by Hering.

Two seminal case reports
David Anderson
The University of Manchester, Manchester, UK.

I live in Umbria and have recently become concerned for the young couple, Amanda Knox and Raffaele Sollecito, convicted of the horrific murder in Perugia on November 1st 2007 of English student Meredith Kercher. They were supposedly acting with an Ivorian, Rudy Guede, who was tried and convicted separately. I am still haunted by a wrongful conviction half a lifetime ago, which involved the murder of 11-year old Lesley Susan Molseed. Just before Christmas 1975, two police inspectors visited me and said they believed the killer was Stefan Ivan Kiszko, a 25-year old Klinefelter’s patient I was treating. They asked casually if such a man could produce sperm. Seven months later I was called to Kiszko’s trial in Leeds, but was never cross-examined. The Defence lawyers wanted him to plead diminished responsibility from testosterone-induced aggression. The vital evidence suppressed by the police was that semen stains on Lesley Molseed’s clothes contained sperm heads. Kiszko always denied the crime, and fourteen years later the reviewing authorities presented me with the suppressed evidence. As a result Kiszko, now schizophrenic, was eventually released; six months later, aged 41, he died of a heart attack. Meanwhile his defence barrister had become Home Secretary; and the prosecuting barrister Lord Chief Justice. The case was described by one MP as “the worst miscarriage of justice of all time”. The real culprit, taxi driver Ronald Castree (strange coincidence that), was after two days of intensive questioning. His conviction was supported by false evidence, exculpatory evidence was ignored, and the tabloid press had a field day. And the cases share willful legal seminal omissions.

The art of medicine
T Toft
Royal Infirmary Edinburgh, Edinburgh, UK.

For the last 15 years, I have suffered from the respectable addiction of collecting modern Scottish art. During that same period I have become alarmed by the many influences which have conspired, not always by chance, to reduce the
The endocrinology of extramarital affairs
Gavin Vinson
School of Biological and Chemical Sciences, London, UK.

There are three types of academic research studies into the origins and maintenance of monogamous as opposed to promiscuous relationships in humans – economic, sociological, and biological – with seemingly little continuity between them. It may be obvious that all three influences can modify sexual behaviour, but it is a fact that though many reproductive strategies exist throughout the animal kingdom – and monogamy is perhaps surprisingly well represented in all groups – relatively few species have the capacity for ‘facultative’ monogamy seen in humans. It seems important to understand the reasons for it, in biological, and also in specifically endocrinological terms. Among the hormones thought to be involved in pair bonding behaviours, prolactin, vasopressin, and above all testosterone have received attention. Testosterone levels are relatively high in promiscuous males, and several studies have shown that they are reduced in monogamous relationships, triggered apparently by proximity to young children. The task is now to identify the causes of this. One possibility is that the effect is pheromonal, and although previous attempts to identify human pheromones have not uniformly stood the test of time, a recent study claims that a pheromone in teardrops has a testosterone reducing effect. There are undoubted rewards to be gained from elucidation of these mechanisms. There is apparently a huge market for Viagra. Is there similarly a demand for an agent with the reverse activity?
Oral Communications
Young Endocrinologists prize session

OC1.1 Thyroid hormones in the euthyroid range predict subsequent body mass composition in women: the OPUS study
Preethi Rao1, Graham Williams2, Elaine Murphy3, David Reid4, Christian Roux5, Dieter Felsenberg2, Claus Gluer6, Richard Eastell6 & Sinclair Rafferty1
1Queen Elizabeth Hospital, Gateshead, UK; 2Molecular Endocrinology Group, Department of Medicine, and Medical Research Council, Imperial College London, Clinical Sciences Centre, London, UK; 3Division of Applied Medical School, Medicine of Dentistry, University of Aberdeen, Aberdeen, UK; 4Universitätsklinikum Schleswig-Holstein, Kiel, Germany; 5Free University of Berlin, Berlin, Germany; 6Paris Descartes University, Paris, France; 7University of Sheffield, Sheffield, UK.

Background
Thyroid disease is associated with BMI with hyperthyroidism causing weight loss and hypothyroidism leading to weight gain. However, the relation of body weight distribution to thyroid function in euthyroid individuals is unclear. This study assessed body composition in euthyroid women.

Methods
Euthyroid women (n = 1072) from the Osteoporosis and Ultrasound Study (OPUS), a population based cohort study recruited participants from 5 European cities, had their thyroid function measured in 2001/02. Individuals with history of thyroid illness, those on any drugs influencing thyroid metabolism and FT3 concentrations <2.1 pmol/l, as a marker of non-thyroidal illness were excluded. Whole body adiposity and lean mass was measured in 2007/08 by dual energy X-ray absorptiometry (DXA). The association between body composition and thyroid function was assessed using correlation analyses (Pearson’s r for FT3 and FT4, and Spearman’s rho for TSH) and linear regression analyses after correction for other baseline confounding variables such as age, BMI, smoking and diabetes.

Results
The mean age (s.d.) was 61 (13) years. Median (range) TSH levels were 0.86 (0.25–3.48) mIU/l, mean FT4 and FT3 levels were 12.8 (1.8) pmol/l and 3.7 (0.84) pmol/l respectively. Whole body and truncal lean mass were negatively correlated with FT3 (r = 0.16 and 0.19), FT4 (r = −0.18 and −0.21) and positively correlated with TSH (p = 0.09 and 0.11). Whole body and truncal fat mass were positively correlated with FT3 (r = 0.09 and 0.12) but not with FT4 and TSH levels. In multiple linear regression analysis, FT3 and TSH independently predicted whole body and truncal lean mass whereas whole body and truncal fat mass were predicted by FT3 alone. FT4 to FT3 ratio was also significantly correlated with truncal fat levels (r = 0.09, P = 0.002).

Conclusions
Thyroid hormone levels, even in the euthyroid range, independently predict whole body lean and fat mass as well as central adiposity. This suggests that thyroid hormones play an important role in the pathogenesis of obesity.

OC1.2 Does ‘mild’ primary hyperparathyroidism progress if left untreated?
A natural history study
Ning Yu, Peter Donnan, David Smith & Graham Leese
University of Dundee, Dundee, UK.

The prevalence of primary hyperparathyroidism (PHPT) is increasing and the majority (over 85%) are now asymptomatic and remain untreated. In order to know whether or not they can be left safely without surgery, issues on disease progression need to be addressed. We aimed to update the natural history of PHPT, with a focus of serum calcium progression in mild untreated patients, selected from a large pre-defined cohort of PHPT in Tayside, Scotland. Possible predictors of progression and the cure rate and outcomes of parathyroidectomy (PTX) were also addressed, corresponding to the Proceedings of the Third International Workshop on asymptomatic PHPT (2008). Using complete medical data at individual level, we identified 904 mild untreated patients (baseline calcium: 2.62 mmol/l) and 200 surgically treated patients (2.80 mmol/l). Their biochemical indices were followed up until September 2009, giving a median follow-up time of 4.7 and 5.8 years respectively. In patients with untreated PHPT, there was a decreased trend in calcium but an increasing trend in PTH for up to 12 years. Serum creatinine, alkaline phosphatase and cholesterol fluctuated around the normal ranges with no apparent trend. Disease progression, defined as an increase in calcium concentration, was observed in 121 patients (13.4%). Baseline and PTX single and the baseline risk factors for disease progression. In comparison, in the PTX group, serum calcium was normalised after surgery in 196 patients (98%). Hospital admissions for renal stones and renal failure were reduced after successful surgery (P < 0.01 in both instances) but not on cardiovascular disease, osteoporotic fractures or cancer. This observational study provides long-term results of treated and untreated PHPT and suggests that ~15% patients with mild untreated PHPT showed evidence of progression. PTH concentration was an important predictor of calcium progression.

OC1.3 Chronically elevated PTH impairs insulin signaling in the heart
Johanna G Maquet1, Carolina S Martinez1, Jorge F Giann1, Lorena Gonzalez1, Ana I Sotel1, Fernando P Dominc1, Andrzej Barlke2 & Daniel Turyn1
1Department of Biological Chemistry, School of Pharmacy and Biochemistry, University of Buenos Aires, IQUIFIB, CONICET, CABA, Buenos Aires, Argentina; 2Southern Illinois University School of Medicine, Springfield, Illinois, USA.

GHI and IGF1 play an important role in cardiac development and function. Conditions leading to abnormal GH levels are associated with cardiovascular disease. Although acromegaly is associated with hypertension, insulin resistance, diabetes type 2 and dislipidemia, several studies ascribe the heart alterations displayed by these patients to the direct action of chronically elevated GH and IGF1 levels.

Given that insulin acts as a growth factor to the heart and is important to cardiac function and considering that GH excess induces hyperinsulinemia, insulin resistance and cardiac alterations, it is of interest to analyze insulin sensitivity in this issue under conditions of chronic GH excess. Thus, in this study we used transgenic mice over-expressing GH (Tg) that develop concentric cardiac hypertrophy associated with alterations in heart functionality, hyperinsulinemia and insulin resistance.

Mice were anaesthetized, received a bolus insulin injection via cauda vein and the heart was removed after 2 min. Tg animals presented cardiomegaly and mild perivascular fibrosis in the heart. The activation and abundance of insulin signaling mediators were assessed by immunoblotting. Insulin-induced tyrosine phosphorylation of the insulin receptor (IR) was conserved in Tg mice compared with their normal littermates. However, the phosphorylation of IR substrate-1 (IRS1), its association with the regulatory subunit of the phosphatidyl inositol 3′ kinase and the phosphorylation of Akt was 25, 50 and 40% decreased, respectively, in Tg mice (P < 0.05, n = 5). Contrary to what was detected in normal animals, insulin failed to stimulate the phosphorylation of mitogen activated protein kinases Erk1/2 in Tg mice. Protein content of the signaling mediators in study was not affected by either hormone stimulation or genotype. We conclude that GH over-expressing Tg mice exhibit decreased sensitivity to insulin at several signaling steps downstream the IR in the heart. These may be associated with the cardiac pathobiology observed in these animals.
Parallel experiments were conducted using 2D cell monolayers. Hyaluronic acid (HA) synthesis was measured using ELISA.

Results

Disregulation of numerous IGF1 signalling genes, including: IGF1, IGFBP6, SOCS3, IRS2, SGK and c-JUN were identified and validated with qPCR. HA synthesis was increased in the presence of both rIGF1 and GO patient serum. HA synthesis was significantly attenuated in the presence of IGFB1R antibody. Corresponding changes in gel thickness were found to correlate with HA concentrations measured with ELISA.

Conclusions

Our microarray data is the first data to demonstrate abnormal IGF1 signalling in human GO tissue and our in vitro model for tissue expansion may be of use as a functional assay for testing potential new treatments and accelerate the move to clinical trials.

OC1.5

Atheroprotection by 11β-HSD1 deficiency in ApoE−/− mice: role of both glucocorticoid and 7-oxysterol factors

Tijana Mitic, Patrick W F Huoake, Surawee Chuaephichai, Taq Y Man, Eileen Miller, Ruth Andrew, Brian R Walker, Karen E Chapman & Jonathan R Seckl
Queen’s Medical Research Institute, University/BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, Scotland, UK.

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) regenerates active glucocorticoids thus amplifying their intracellular actions. 11β-HSD1 deficiency or inhibition, which improve metabolic syndrome and attenuate atherosclerosis in vulnerable rodent strains, is a target for drug development. However, 11β-HSD1 also converts 7-ketocholesterol (7KC) which accumulates in fatty tissues, to cholesterol ‘Western’ diet and saline (0.9%) for 12 weeks. The aorta and branches were perfusion-fixed. Lesion volume and extracellular lipids were determined by 3D optical projection tomography (OPT) and plasma and lesion lipid profiles by colorimetric assays and gas chromatography mass spectrometry. Body/organ weights were unaltered by adrenalectomy in either genotype. Adrenalectomy in ApoE−/− mice did not alter lesion volume (232 ± 24 vs 235 ± 34 µm3 sham control). DKO mice had reduced lesion volumes (139 ± 17 µm3) compared with ApoE−/− mice (P < 0.05). Adrenalectomy reversed this effect (263 ± 52 µm3). DKO mice (both sham and Adx) had increased plasma levels of 7KC (66 ± 8 mg/ml vs 23 ± 23 DKO Adx) compared with ApoE−/− (48 ± 8 ng/ml; 36 ± 9 ApoE−/− Adx). Whilst aortic cholesterol levels were unchanged by adrenalectomy, aortic 7KC levels, expressed as a ratio to total plasma cholesterol, were increased in adrenalectomised DKO mice (51.2 ± 13.8 Adx versus 14.9 ± 3.3 sham) but remained unaltered in ApoE−/− mice (18.9 ± 3.7 vs 19.6 ± 3.8). Circulating adrenal products, possibly glucocorticoids, are therefore necessary for 11β-HSD1 deficiency to attenuate atherosclerosis. However, 11β-HSD1 deficiency increases the lipid content of plaques in the absence of glucocorticoids, perhaps due to accumulation of 7-ketocholesterol. Thus metabolism of both glucocorticoids and 7-oxosterols by 11β-HSD1 appear involved in atherogenesis.

OC1.7

Mutant cytochrome b5 causing 46,XY disorder of sex development (DSD) due to apparent CYP17A1 17,20 lyase deficiency

Jan Idkowiak1, Tabitha Randell1, Vivek Dhir1, Pushpa Patel1, Cedric H L Shackleton1, Nils Kron1 & Wiebke Arlt1
1School of Clinical and Experimental Medicine, CEDAM Centre for Endocrinology, Diabetes and Metabolism, University of Birmingham, Birmingham, UK; 2Department of Paediatric Endocrinology, Nottingham Children’s Hospital, Nottingham University Hospitals NHS Trust, Nottingham, UK.

In humans, androgen synthesis crucially depends on the enzyme CYP17A1 expressed in adrenals and gonads. The 17,20 lyase activity of CYP17A1 catalyses the key step in human androgen biosynthesis, the conversion of 17-hydroxyprogrenolone to the universal sex steroid precursor dehydroepiandrosterone (DHEA). For its catalytic activity, CYP17A1 requires electron transfer from P450 oxidoreductase (POR) and homologous OR mutants A287P and H262P are associated with 46,XX DSD and 46,XY DSD, respectively, and we hypothesised that this is explained by distinct effects on alternative androgen pathway activity. In vitro yeast microsomal co-expression of CYP17A1 with both mutants revealed 65% residual conversion of 5α17THP to An in the presence of A287P whereas co-expression of H262P maintained only 16% of wild-type activity. Longitudinal urinary steroid profiling in DSD due to A287P (n=3) in comparison to controls (n=8) documented significantly increased 5α17HP and An excretion during the first three weeks of life. To determine whether there is also evidence for the alternative pathway in normal physiology we performed ex vivo fetal organ culture employing tissues from gestational weeks 7 to 9, the key period of human sexual differentiation, with steroid identification and quantification by tandem mass spectrometry. Results unanimously showed that the human fetal adrenal is capable of all conversions within the proposed alternative pathway, except for the final step, the generation of DHT from androstenediol, which occurred in fetal genital skin. Our results provide conclusive evidence for the existence of an alternative androgen biosynthetic pathway in early human life, with major implications for human physiology and disease.
Objective Hypothyroidism is common and predominantly managed in primary care. Symptoms are non-specific, with thyroid function tests (TFT) required for diagnosis. We sought to investigate current practice in levothyroxine prescribing in primary care.

Methods
We studied the initiation of levothyroxine using the General Practice Research Database (GPRD), the world’s largest database of anonymised medical records. Individuals with i) thyroid cancer ii) secondary hypothyroidism iii) other thyroid altering medication were excluded. This was combined with a study of 444 patients (DEPTH) referred for thyroid function testing from 5 general practices and a detailed records-based analysis of the reasons for commencing levothyroxine in 104 subjects from one general practice.

Results
Fourteen thousand and nine eligible patients prescribed levothyroxine between 2000 and 2009 were identified from GPRD. The median TSH at which levothyroxine was initiated was 6.0 mU/l. 78.3% of individuals had levothyroxine prescribed at a TSH level < 10 mU/l and 36.2% at < 5 mU/l. The odds ratio (OR) for psychological caseness amongst patients in DEPTH was 1.70 (95% CI 0.87–0.87). Detailed records analysis of 104 subjects from one general practice revealed 43.3% had levothyroxine prescribed for other ‘non-prescribed due to ‘classical’ (cold intolerance, weight gain, thin hair) symptoms.

The mechanism by which expression of 11β-HSD1 is regulated in stromal cells appears distinct from that reported in non-stromal cells such as hepatocytes. The finding that stromal cell expression of 11β-HSD1 activity is regulated by NF-κB opens up new opportunities to inhibit 11β-HSD1 activity in a tissue restricted manner.
associated with an increased plasma aldosterone levels and hypertension. However, deletion of this site has no effect on gene transcription in vitro and therefore the mechanism that links genotype with phenotype is unclear.

We identified a polymorphism at position −1651 T/C (rs13286025) binding show it to be in linkage disequilibrium with the −344 SNP. Reporter gene constructs containing contrasting alleles at position −1651 were transfected into H295R cells. The C allele had (75%) greater promoter activity than the T allele. An electromobility shift assay with oligonucleotides spanning the polymorphism of interest demonstrated a difference in protein/DNA complexes between the T and C allele. Biotinylated pull down assays of the protein: DNA complex were proteolysed and peptides identified by tandem mass spectrometry. Fragments derived from the transcription factor APEX1, were identified bound to the T allele and not the C allele. Chromatin immunoprecipitation confirmed the association of APEX1 to the CYP11B2 promoter. Inhibition of APEX1 activity with a small molecule inhibitor of APEX1 (E3330, Sigma–Aldrich), and siRNA for APEX1, both increased transcriptional activity. The in vitro genotype/phenotype relationship was explored in 60 normal volunteers, studied under standard salt intake. Carriers of the C allele were confirmed to have higher 24 h urinary aldosterone excretion (mean ± SEM: 59.55 ± 22.4 h 95% CI 46.73–72.37 µg/24 h) than the subjects carrying the T allele at position −1651 (mean ± SEM: 36.10 ± 22.4 h 95% CI 24.0–48.21 µg/24 h) P=0.008.

APEX1 has been identified as a novel negative regulator of CYP11B1B; a SNP at −1651 demonstrates allele dependant binding, leading to increased transcriptional activity in vitro and increased THAldo excretion in vitro. This offers a plausible mechanism behind the genotype/phenotype association of increased aldosterone secretion and hypertension.

### OC2.4

**Altered miR-125 and miR-134 expression in aldosterone-producing adenoma and post-transcriptional regulation of the CYP11B2 gene**

Stacy Wood1, Ayesha Ejaz1, Craig Livie1, Scott MacKenzie1, John Connell2

& Eleanor Davies3

1University of Glasgow, Glasgow, UK; 2University of Dundee, Dundee, UK.

Essential hypertension is known to have a large genetic component. Variation in the CYP11B2 gene, which encodes the aldosterone synthase enzyme, is associated with excess aldosterone production and hypertension but the causative mechanism remains elusive. miRNAs are a class of post-transcriptional regulatory molecules, implicated in cardiovascular disease, development and tumourogenesis. They act by targeting the 3’ untranslated region (UTR) of mRNAs, inhibiting translation through mRNA cleavage or destabilisation. We propose a role for miRNAs in the regulation of CYP11B2 expression and in the development of essential hypertension. To study this, we characterised the mRNA profile of four normal adrenal glands and four aldosterone-producing adenomas (APA), which excrete excess aldosterone and cause hypertension. We then investigated the effect of four miRNAs (miR-125a-5p, miR-125b, miR-134 and miR-495a) which are expressed in these tissues and using bioinformatics we predicted to bind the 3’UTR of CYP11B2 mRNA.

Comparison of mRNA expression levels showed that miR-125a-5p and miR-134 are significantly down-regulated in APAs. The interaction of all four miRNAs with the CYP11B2 3’UTR was tested using a PEZX-reporter plasmid containing the full-length CYP11B2 3’UTR sequence. This was co-transfected into HeLa cells alongside a synthetic miRNA (pre-miR) or miRNA inhibitor (anti-miR). Both miR-125a-5p and miR-125b pre-miRs significantly decreased luciferase activity when over-expressed. Conversely, their anti-miRs significantly increased luciferase activity. This is consistent with canonical miRNA binding and repression, confirming our bioinformatic predictions. miR-495a and miR-134 pre-miRs and anti-miRs did not alter luciferase activity significantly.

In summary, we have identified two miRNAs, miR-125a-5p and miR-134, with aberrant expression in APA samples and putative binding sites in the CYP11B2 gene. We also confirmed that miR-125a-5p and miR-125b, but not miR-134, can repress CYP11B2 by directly targeting the 3’UTR. The regulation of CYP11B2 mRNA by miRNAs and altered expression in adrenal adenomas may give rise to novel therapeutic targets for the treatment of essential hypertension and adrenal tumours.

### OC2.5

**A novel entity of isolated adrenal insufficiency caused by partial inactivation of P450 side-chain cleavage (CYP11A1) enzyme**

Silvia Parajes1, Clemens Kamrath2, Ian Rose3, Angela Taylor4, Christiaan Mooij2, Vivek Dhir5, Joachim Grotzinger5, Wiebke Artl6 & Nils Krone6

1University of Birmingham, Birmingham, UK; 2University Children’s Hospital Giessen, Giessen, Germany; 3University of Kiel, Kiel, Germany.

Cytochrome P450 side-chain cleavage enzyme (CYP11A1) catalyses the first and rate-limiting step of steroidogenesis, facilitating conversion of cholesterol to pregnenolone. Cholesterol, transported by steroidogenic acute regulatory protein (StAR) into the inner mitochondrial membrane, is converted by CYP11A1 into 22R-hydroxysterol. Subsequently, CYP11A1 converts 22R-hydroxysterol by 20alpha-hydroxylisation and cleavage of the C20-C22 bond into pregnenolone. All patients with CYP11A1 deficiency described so far presented with combined adrenal insufficiency (AI) and 46,XY disorder of sex development (DSD).

Herein, we describe two siblings (46,XY) presenting with late-onset isolated AI with normal male genital development. The older sibling had first symptoms suggestive of AI at the age of 2 years with the diagnosis finally established at the age of 4.1 years. His younger brother was diagnosed with adrenal insufficiency at the age of 2.5 years. A maternal uncle died at the age of 9 years during an episode of febrile seizures.

Mutation analysis of the DAX1 and StAR genes was performed but did not reveal disease-causing mutations. Finally, we detected a novel homozygous CYP11A1 mutation (c.135C>T; p.Arg45Trp) in both siblings. Segregation analysis confirmed that both parents were heterozygous carriers. Functional in vitro analysis was performed in COS7 cells co-transfected with wild-type or mutant CYP11A1, with or without wild-type STAR and adrenodoxin. Transfected cells were incubated with either cholesterol or 22R-hydroxycholes- terol and pregnenolone production was measured by liquid chromatography/tandem mass spectrometry (LC-MS/MS). This demonstrated partial inactivation of CYP11A1 activity by the p.Arg45Trp mutation consistent with results of in silico analysis.

For the first time, mutant CYP11A1 enzyme activity has been assessed in vitro employing the natural substrate cholesterol and the intermediate product 22R-hydroxycholesterol using LC-MS/MS. Importantly, we present a novel entity of isolated AI caused by a partially inactivating CYP11A1 mutation, which should be considered as differential diagnosis in patients with unexplained isolated adrenal insufficiency.

### OC2.6

**Novel non-steroidal glucocorticoids that dissociate rapid signalling effects from gene transcription**

Peter Trebble1, Karen Simpson1, Laura Matthews1, Stuart Farrow2 & David Ray

1Endocrinology and Diabetes, Developmental Biomedicine Research Group, University of Manchester, Manchester, UK; 2Inflammation and Resolution Group, Respiratory CEDD, GlaxoSmithKline, Stevenage, UK.

Glucocorticoids (GCs) are used extensively in the treatment of inflammatory disease. Unfortunately, as GC act on virtually every organ system they also carry a broad range of serious side-effects which limits their clinical use. The structure of the bound GC strongly impacts the final conformation of the glucocorticoid receptor (GR) and thereby dictates downstream events. Designing drugs with different GR binding properties therefore offers a plausible route to achieve dissociative effects on GR action. This work describes two novel, high-affinity, high specificity, non-steroidal GCs (NSGs). Both NSGs were more potent than the synthetic GC dexamethasone for gene transactivation and transrepression and were equipotent for inhibition of cell proliferation. Compared with dexamethasone, short treatment with the NSGs induced significantly less phosphorylation of PKB, which was accompanied by delayed, submaximal phosphorylation of GR at Ser211. Ser211GR is required for engagement of GR at some (eg IGFBP1) but not all gene targets, suggesting that the NSGs may mediate only a proportion of GR effects. Indeed, compared with dexamethasone, the NSGs showed impaired transactivation of IGFBP1.
Perhaps most surprisingly, the NSGs failed to promote downregulation of steady state GR expression, a well characterised response to dexamethasone. Previous reports have demonstrated that GSK3β, a downstream target of PKB, is important for targeting the GR for degradation by the proteasome. As the NSGs also fail to induce rapid activation of PKB, this provides a link between initiation of rapid kinase signalling events and GR turnover.

These novel NSGs therefore do not activate the full spectrum of rapid GC effects, impacting kinase signalling, GR activity and turnover. As a consequence the NSGs show an unexpected specificity of action. In particular, this highlights the requirement of rapid signalling events to mediate the full range of GC effects, representing an important and tractable model for drug design.

Depot specific differences in the sensitivity to glucocorticoid and insulin action in human adipose tissue
Laura Gathercole, David Hauton, Stuart Morgan, Iwona Bujalska, Paul Stewart & Jeremy Tomlinson
University of Birmingham, Birmingham, UK.

Intra-abdominal adiposity is associated with insulin resistance and increased cardiovascular morbidity and mortality. Differences in gene expression between omental (om) and subcutaneous (sc) adipose have been described, but molecular mechanisms underpinning differences in adipose biology are not known. Patients with glucocorticoid excess, Cushing’s syndrome, develop a phenotype characterized by central obesity. We have characterized the regulation of lipogenesis by glucocorticoids and insulin in paired om and sc differentiated pre-adipocytes measuring lipogenic gene expression by real-time PCR and 1-[14C]-acetate incorporation into lipid.

Differentiated sc pre-adipocytes had more lipid droplets, increased mRNA expression of lipogenic genes (ACC1 3.2 fold, FAS 10.9 fold, ACC2 17.8 fold, LPL 44.7 fold) and higher 1-[14C]-acetate incorporation than om cells (8.9 fold). However, lipogenesis in om cells was more responsive to insulin stimulation (control 1.86%; sc 149.2±22.14%, om 209±1.86%, P<0.05). In the absence of insulin, Dexamethasone (Dex) had no effect on lipogenic gene expression in sc or om cells. However, in the presence of insulin, in both sc and om cells, Dex increased FAS expression (sc ± 2.4 fold; om 3 fold) and in om cells only, increased ACC1 expression (1.6 fold). Dex decreased lipogenesis in sc (100% control), 81.9±3.9% (5 nM), 67.3±4.8% (500 nM)) and om (72.0±5.4% (5 nM), 46.9±5.7% (500 nM)) cells in a dose dependent manner. In sc cells, in the presence of insulin (5 nM), Dex no longer inhibited lipogenesis and did not impair insulin-stimulated lipogenesis (100% control), 149.0±22.1% (No Dex), 145.5±29.4% (5 nM), 128.2±39.3% (500 nM)). In contrast, Dex decreased lipogenesis in om cells in the presence of insulin, reflecting either a persistence of the direct action of Dex, or its ability to induce insulin resistance and limit insulin-stimulated lipogenesis (100% control), 208.9±1.86% (No Dex), 181.45±11.8% (5 nM), 125.1±12.0% (500 nM)).

Om adipose tissue sensitivity to both glucocorticoids and insulin may drive lipid flux, contributing to the Cushing’s phenotype and may also help to explain the detrimental impact of om fat accumulation.

Five obese men (age 48±5 years, BMI 39.8±3.6 kg/m²) participated in a randomised, double-blinded, crossover study comparing metformin (1 g BD orally for 28 days) with placebo. At the end of each phase, subjects collected a 24 h urine sample and attended twice after overnight fast. On a first visit, 9,11,12,13-[3H]cortisol (d4-cortisol) was infused for 4 h, with repeated blood sampling to measure steady state d3-cortisol appearance (whole body 11b-HSD1 activity). On a second visit, subjects took 0.25 mg oral dexamethasone at 2300 h and 25 mg oral cortisol at 0900 h, with repeated blood sampling to measure conversion of cortisol to hepatic 11b-HSD1. Steroids were quantified by RIA (cortisone test) or mass spectrometry (tracer and urinary steroids). Local ethical approval was obtained. Data are mean±S.E.M.

Fasting glucose (5.3±0.3 vs 6.0±0.9 mmol/l) and insulin (11.4±3.5 vs 21.0±7.8 mU/l) were non-significantly decreased by metformin. Metformin increased whole body rate of appearance of d3-cortisol (48±6 vs 39.5±5 mmol/min, P<0.01), but did not alter the rate of conversion of oral cortisone to cortisol (area under curve 29 951±11 207 vs 34 128±3 741, P=0.7) or urinary cortisol metabolites (15.9±4.0 vs 18.3±4.2 mg/day).

Metformin increases whole body 11b-HSD1 activity in euglycaemic obese men. However, whether this is mediated in the liver by reversal of hyperinsulinaemia remains unproven. There has been concern that 11b-HSD1 inhibitors will be less effective in the presence of metformin, given their shared mechanism of suppressing hepatic gluconeogenesis. These data suggest, however, that metformin may increase the local regeneration of cortisol by 11b-HSD1 and provide a bigger target for 11b-HSD1 inhibitors.

Development of a novel mass spectroscopy-based method for determining serum IGF1: assessment in a cohort of newly diagnosed subjects with acromegaly
David Halsall1, Richard Kay2, Kevin Taylor3, Anand Annamalai4, Narayanand Kandasamy5, Gwen Wark6, Steve Pleasance7 & Mark Garnell8
1Addenbrookes Hospital, Cambridge, UK. 2Quotient Bioresearch Ltd, Fordham, Cambridgeshire, UK. 3Royal Surrey County Hospital, Guildford, UK.

Background
The recently published ‘Consensus on Criteria for Cure of Acromegaly’ (Giustina et al. ICEM, 2010) highlighted concerns regarding the quality of currently available insulin-like growth factor 1 (IGF1) immunoassays which may contribute, at least in part, to the discordance between GH and IGF1 that is observed in up to 30% of patients with acromegaly after treatment. The development of mass spectroscopy (MS)-based technology has been proposed as a potential solution to these limitations.

Methods
Here, we report the development of a stable isotope dilution ultra-performance liquid chromatography tandem MS (UPLC-MS/MS)-based method for the quantitation of serum IGF1. The method employs selected reaction monitoring (SRM) of two tryptic peptides derived from IGF1, and relies on solid phase extraction for enrichment of the peptide fraction containing IGF1 rather than immunocapture, so is less susceptible to antibody interference. The UPLC separation of the peptides was performed using a C18 column prior to MS/MS analysis on a 5500 QTrap MS. The method is not affected by concentrations of IGBPBP3 up to 420 nmol/l.

Results
The method showed good correlation with an IGF1 immunnoassay (Siemens Immulite 2000) over a wide range of serum IGF1 concentrations (5.4–261 nmol/l by immunnoassay) in a cohort of 45 patients that included 25 subjects with acromegaly, assessed both before and after primary medical therapy. The Passing and Bablock regression was: LC–MS/MS (nmol/l) = 1.4X Immunnoassay (nmol/l) + 4.4. Analysis of UKNEQAS material with an immunnoassay method mean of 22.2 and 45 nmol/l returned values of 22.3 and 47.6 nmol/l respectively.

This method relies on entirely different physicochemical principles to the ubiquitous ‘sandwich immunoassay’ for IGF1, and thus provides an independent validation of suspicious immunnoassay-derived results. A further advantage of the method is that, with the addition of appropriate internal standards, there is potential for extensive multiplexing of serum peptide assays.

Endocrine Abstracts (2011) Vol 25
OC3.2

Determination of method-specific normal cortisol responses to the short Synacthen test
Nadia El-Farhan1,2, Alan Pickett1, David Ducrocq3, Catherine Bailey4, Kelly Parham4, Nicola Morgan5, Anne Armston1, Carol Evans1 & Dafydd Aled Rees2
1University Hospital of Wales, Cardiff, UK; 2Cardiff University, Cardiff, UK; 3Welsh External Quality Assurance Scheme Laboratory, Cardiff, UK; 4Royal Gwent Hospital, Newport, UK; 5Prince Charles Hospital, Merthyr Tydfil, UK; 2Bristol Royal Infirmary, Bristol, UK; 3Southampton General Hospital, Southampton, UK.
Introduction and aims
The short Synacthen test (SST) is used to diagnose underactivity of the HPA axis, with a cortisol response $<$ 550 nmol/l at 30 min widely accepted as diagnostic of adrenal insufficiency. Earlier studies of the SST have shown variation in cortisol concentrations measured by different immunoassays, suggesting the need for method-specific cut-offs. We sought to define the cortisol response to Synacthen in healthy volunteers (HV) and establish method-specific lower reference limits (LRL) for this response.

Methods
R + D, MIHRA and ethical approval were obtained. A SST was undertaken in 165 HV (age 20–66 years, 105 female, 24 taking an oral contraceptive pill (OCP)). Serum cortisol was measured using GC–MS, Siemens (Centaur and Immulite), Abbott, Roche and Beckmann automated immunoassays. The estimated LRL for the 30 min cortisol response to Synacthen was derived from the 2.5th percentile of log-transformed concentrations.

Results
The GC–MS-measured cortisol response was normally distributed in males but positively biased relative to GC–MS, except in samples from women on the OCP. The estimated LRL for cortisol is method-specific, and, for the Roche assay, also gender-specific. A separate LRL is necessary for women on the OCP.

Conclusions
This is the largest study to examine cortisol responses to Synacthen in healthy volunteers and provides clinical laboratories with method-specific estimated lower cortisol limits for the SST. We recommend the use of separate cut-offs in volunteers and provides clinical laboratories with method-specific estimated lower reference limits (LRL) for this response.

Table 1

<table>
<thead>
<tr>
<th>Method</th>
<th>Adults</th>
<th>OCP</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>GC–MS</td>
<td>420.4</td>
<td>649.4</td>
<td>498.7</td>
</tr>
<tr>
<td>Centaur</td>
<td>430.4</td>
<td>682.3</td>
<td>577.0</td>
</tr>
<tr>
<td>Abbott</td>
<td>M 573.5</td>
<td>791.2</td>
<td>F 524.4</td>
</tr>
<tr>
<td>Roche</td>
<td>474.4</td>
<td>687.7</td>
<td>458.6</td>
</tr>
<tr>
<td>Immulite</td>
<td>456.6</td>
<td>603.8</td>
<td></td>
</tr>
<tr>
<td>Beckmann</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OC3.3

miR-107 inhibits the expression of aryl hydrocarbon receptor interacting protein (AIP) and is potentially involved in pituitary tumorigenesis
Gianpamolo Trivellin1, Susana Igreja1, Edwin Garcia1, Harvinder Chahal1, Henriett Butz2, Attila Patocs3, Ashley Grossman1, Christopher McCabe1 & Kristien Boelaert1
1Department of Endocrinology, Bart’s and the London School of Medicine, Queen Mary University of London, London, UK; 2Second Department of Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary; 3Molecular Medicine Research Group, Hungarian Academy of Sciences and Second Department of Medicine, Faculty of Medicine, Semmelweis University, Budapest, Hungary.

Background
Abnormal micro RNAs (miRNAs) expression profiles have been recently associated with sporadic pituitary adenomas, suggesting that miRNAs can contribute to tumour formation. miRNAs are small noncoding RNAs which inhibit post-transcriptional expression of target mRNAs by binding to complementary sequences usually located in the 3’ untranslated region (3’UTR). However, the substantial lack of knowledge about miRNAs’ targets hinder full understanding of the mechanisms by which they influence tumorigenesis.

Methods
The expression level of miR-107 were evaluated in 17 sporadic pituitary adenoma tissues and nine normal pituitary samples using a micro RNA screen TLDA and qRT-PCR analyses. Pre-miR-107 and anti-miR-107 were used to examine the effects of miR-107 expression on cell proliferation and colony formation in human SH-SY5Y and rat GH3 cells. A luciferase reporter assay was used to examine the in silico predicted target sites in the 3’UTR of the aryl hydrocarbon receptor interacting protein (AIP) gene.

Results
miR-107 expression was found significantly upregulated in pituitary adenoma tissues compared to normal pituitaries. Overexpression of miR-107 inhibited cell proliferation of both the rat and the human cell lines tested. Anti-miR-107 increased colony formation by 2.5 fold in human cells. We provide direct evidence that AIP – 3’UTR is a functional target of miR-107 as miR-107 inhibits human AIP expression to 53.9 ± 2% of the scrambled miRNA control in a luciferase expression model and it reduces endogenous AIP expression to 53 ± 22% of the scrambled miRNA control in SH-SY5Y cells.

Conclusions
These results suggest that miR-107 functions as a tumor suppressor gene in neuroblastoma and pituitary adenoma cells and that it may play a role in pituitary adenoma pathogenesis.

OC3.4

PTTG promotes mitogenic mechanisms in thyroid cells through autocrine pathways of interaction with growth factors
Gregory Lewy, Gavin Ryan, Sarah Stewart, Martin Read, Vicki Smith, Jim Fong, Adrian Warfield, Margaret Eggo, Robert Seed, Neil Sharma, Perkin Kwan, Jayne Franklin, Christopher McCabe & Kristien Boelaert
University of Birmingham, Birmingham, UK.

The human pituitary tumour transforming gene (hPTTG) is overexpressed in thyroid cancers; it induces genetic instability and propagates growth through the induction of growth factors. We investigated the pathways of interaction between hPTTG and epidermal growth factor (EGF), transforming growth factor-α (TGF-α), insulin-like growth factor 1 (IGF1) and basic fibroblast growth factor (FGF2) in vitro and in vivo. Synchronised K1 and TPC-1 papillary thyroid carcinoma cells were treated with TGF-α (5 nM), EGF (5 nM), IGF1 (10 ng/ml), or FGF-2 (5 nM) and hPTTG protein expression was determined by western blotting at 24 h. EGF treatment in both K1 and TPC-1 cells induced a 2-fold upregulation of hPTTG, an effect abrogated by treatment with the specific MAPK inhibitor PD98059 (30 μM), but not with the specific PKC inhibitor BIS-I (50 nM). TGF-α treatment upregulated hPTTG in K1 (3.6-fold) and TPC-1 cells (4-fold), with similar abrogation by treatment with PD98059 but not with BIS-I. IGF1 treatment induced hPTTG in K1 (2.3-fold) and TPC-1 cells (2.6-fold), where treatment with either of the specific PI3-kinase inhibitors Wortmannin (20 μM) and LY294002 (50 μM) abrogated this effect. FGF2 did not affect K1 cells but significantly upregulated hPTTG in TPC-1 cells (7-fold). To investigate if hPTTG in turn induces expression of these growth factors, we determined TGF-α, EGF and IGF1 mRNA expression through TaqMan RT-PCR following transient transfection of primary human thyrocytes with hPTTG. EGF (1.7-fold, n=4, P=0.004), IGF1 (1.6-fold, n=5, P= 0.002) and TGF-α mRNA (1.6-fold, n=3, P=0.024) were significantly upregulated by hPTTG. To investigate these findings in vivo, we evaluated mRNA expression of these mitogenic factors in our transgenic mouse model with thyroid-targeted hPTTG overexpression. Upregulation of mIGF1 (2.7-fold, n=5, P=0.012) and mFGF2 (2.0-fold, n=3, P=0.02) was confirmed when comparing 6-week-old hPTTG+/+ mice to age-matched WT mice.

Conclusion
PTTG is involved in autocrine signalling mechanisms with growth factors including TGF-α, EGF, IGF1 and FGF2 in the thyroid. Ablation control of these pathways may enhance tumour development and further elucidation of these pathways may provide novel therapeutic targets for the prevention of thyroid tumour progression.

OC3.5

Metastatic characteristics and radiosensitivity of thyroid carcinoma cells depends on HIF-1 and PI3K signalling in vitro and in vivo
Natalie Burrows1, Muhammad Babur2, Julia Resch3, Sophie Ridsdale1, Joseph Williams1, Georg Brabant4 & Kaye Williams3
1School of Pharmacy, University of Manchester, Manchester, Lancashire, UK; 2Department of Endocrinology, Christie Hospital, Manchester, Lancashire, UK.

The transcription factor HIF-1α regulates hypoxia-mediated gene expression which promotes tumour growth, metastasis and desensitisation to chemo-and
radiotherapy. HIF-1 is functional in a range of thyroid-carcinoma cells and is regulated by hypoxia and the PI3K and MAPK-signalling pathways. We aimed to determine if HIF-1 and PI3K were important in the development of a metastatic phenotype and play a role in desensitisation to radiotherapy in thyroid carcinomas.

Thyroid cell migration and adhesion were monitored as metastatic characteristics in vitro. Migration increased with decreasing oxygen tension and was inhibited by i) the PI3K-inhibitors PI-103 and GDC-0941; ii) reintroduction of PTEN into PTEN-null FTC133 cells and iii) inhibiting HIF-1 function using a dominant negative protein (dnHIF) that reduced expression of HIF-1-target genes CA-9, LDH-A, GLUT-1, VEGF. PTEN-expressing FTC133s, dnHIF-FTC133s and cells treated with GDC-0941 also had reduced ability to adhere/spread on fibronectin. PI-103 and GDC-0941 inhibited expression of PI3K target proteins (pAKT, pGSK-3β) and HIF-1 target genes. Radiation induced HIF-1 expression. GDC-0941 combined with radiation blocked HIF-1 induction, prolonged DNA double-strand breaks and reduced clonogenic survival suggesting a radiosensitising effect.

In vivo, PI-103 and GDC-0941 reduced PI3K and HIF-1α activity and downstream target genes in follicular and anaplastic tumours. Mice bearing dnHIF-FTC133 tumours and those treated with GDC-0941 had decreased metastatic colonies in the lungs. FTC133, dnHIF-FTC133 and 8505c cells were manufactured to express eGFP. Spontaneous metastatic colonization to the lungs was confirmed by viewing eGFP-positive colonies grown from digested lung tissue and by immuno-blotting for eGFP.

These data link PI3K, HIF-1-activation and aggressive disease in thyroid carcinoma. With the known desensitising effects of HIF-1 and PI3K activity on radiation treatment, a combined approach involving both radiation and a PI3K inhibitor may improve both the therapeutic response within the tumour and reduce radiation treatment, a combined approach involving both radiation and a PI3K inhibitor may improve both the therapeutic response within the tumour and reduce metastatic potential in thyroid carcinomas. This is a focus of ongoing studies.

Morbidity in patients with endogenous subclinical hyperthyroidism

Thennmal Vadiveloo, Peter Donnan, Lynda Cochrane & Graham Leese

University of Dundee, Dundee, UK.

Objective
To investigate the long term outcomes for patients with endogenous subclinical hyperthyroidism (SH).

Design
Population record-linkage technology was used retrospectively to identify patients with SH and hospital admissions from January 1, 1993 to December 31, 2009.

Patients
All residents over 18 years old with at least two serum TSH measurements below the reference range for at least 4 months apart and normal free T4/total T4 and normal total T3 concentrations at baseline were included as potential cases. Using a unique patient identifier, data-linkage enabled a cohort of SH cases to be identified from various medical records matched to five comparators from the general population.

Outcome measures
Cardiovascular disease, fracture, dysrhythmia, dementia and cancer based on ICD 10 codes.

Results
Compared to the reference population, SH was associated with an increased risk of non-fatal cardiovascular morbidity, osteoporotic fracture, dysrythmia and dementia: adjusted hazard ratios (HR) 1.39 (1.22–1.58), 1.25 (1.04–1.50), 1.65 (1.26–2.17) and 1.64 (1.20–2.25) respectively. When SH patients who developed overt hyperthyroidism during follow-up were excluded, SH patients were associated with an increased risk of cardiovascular morbidity (HR 1.36 (1.19–1.57), dysrythmia (HR 1.39 (1.02–1.90)) and dementia (HR 1.79 (1.28–2.51)), but not fracture and cancer.

Conclusion
Patients with endogenous SH might have an increased risk of cardiovascular disease and dysrythmia. There is an association with fracture and dementia that is not related to TSH concentration. No association was found between SH and cancer.

OC3.7
Thyroid dysfunction and clot structure: a mechanism for increased thrombotic risk in hyperthyroid individuals?

Jonathan Hooper1, Steve Orme2, Bregje van Zaane3, Katharina Hess3, Saad Alzahrani4, Kristina Standeven5, Simon Pearce5, Peter Grant5 & Ramzi Ajjan6

1Division of Cardiovascular and Diabetes Research, Leeds Institute of Genetics, Health and Therapeutics, University of Leeds, Leeds, West Yorkshire, UK; 2Department of Endocrinology, Leeds Teaching Hospital NHS Trust, Leeds General Infirmary, Leeds, West Yorkshire, UK; 3Department of Internal Medicine, Slotervaart Hospital, Amsterdam, The Netherlands; 4Institute of Human Genetics, International Centre for Life, Newcastle-upon-Tyne, UK.

Hyperthyroidism is associated with increased thrombotic risk through mechanisms that are not fully understood. In contrast, a tendency to bleeding has been reported in hypothyroidism. Fibrin network structure has been shown to determine susceptibility to atherothrombosis and venous thrombotic events and therefore, this study investigates the effects of thyroid-dysfunction on clot structure and fibrinolysis using longitudinal interventional studies.

Seventeen hyperthyroid patients were recruited (mean age 39.41 ± 2.6) and treated with antithyroid medication until euthyroid. Twenty hyperthyroid subjects were also recruited (mean age 49.6 ± 14.8) and treated with i-131. In both groups, blood samples were taken at baseline and after normalisation of thyroid function.

Seventeen hyperthyroid patients were recruited (mean age 39.41 ± 2.6) and treated with antithyroid medication until euthyroid. Twenty hyperthyroid subjects were also recruited (mean age 49.6 ± 14.8) and treated with i-131. In both groups, blood samples were taken at baseline and after normalisation of thyroid function.

Coagulation-fibrinolysis characteristics were investigated ex vivo using a validated turbidimetric assay and the following parameters studied: i) lag phase (Lp): indicating thrombogenic tendency, ii) maximum absorbance (MA) measuring clot density, iii) time to 50% lysis (L50), assessing fibrinolytic potential, iv) Lysis area (LA), measuring the balance between clot formation and lysis.

Plasma levels of fibrinogen, plasminogen activator inhibitor-1 (PAI-1), C-reactive protein (CRP) and complement C3 were also measured.

Lp was 612 ± 21.1 in hyperthyroid subjects, increasing to 709 ± 23.9 after normalising thyroid function (P = 0.001), whereas MA fell from 0.43 ± 0.03 to 0.36 ± 0.02 a.u. (P = 0.009). LA was larger at baseline than post treatment (246 ± 26.4 and to 164 ± 22, respectively; P = 0.002). Fibrinogen, PAI-1 and C3 levels were all higher in the hyperthyroid phase. In contrast, hyperthyroid subjects had longer Lp at baseline compared with post-normalisation of thyroid function (354 ± 13 and 321 ± 7 s, respectively; P = 0.005), whereas MA increased from 0.32 ± 0.02 to 0.36 ± 0.02 (P = 0.008).

Hyperthyroid patients have a procoagulant tendency, with short lag phase, compact clot structure and impaired-lysis, whilst the opposite is true in hypothyroidism. We suggest that altered clot structure in hyperthyroid subjects is responsible, at least in part, for increased thrombotic risk in this population.

Endocrine Abstracts (2011) Vol 25
Bone and diabetes

**OC4.1**

A mouse with an ENU-induced mutation (Tyr209Asn) in the natriuretic peptide receptor 3 (Npr3) develops autosomal recessive kyphosis

Christopher Šapka1–2, Rosie Head1,2, Gethin Thomas1, Matthew Brown1, Peter Croucher1, Roger Cox2, Steve Brown2 & Rajesh Thakker1

1University of Oxford, Oxford, Oxfordshire, UK; 2MRC Harwell, Harwell, Oxfordshire, UK; 3University of Queensland, Brisbane, Queensland, Australia; 4University of Sheffield, Sheffield, UK.

Kyphosis is a common spinal disorder affecting up to 8.3% of the population, and associated with significant morbidity. Familial and twin studies have implicated a genetic involvement. However, the causative genes have not been identified. Studies investigating the underlying molecular mechanisms are hampered by genetic heterogeneity, small families and variable modes of inheritance displayed by different kindreds. To overcome these limitations, we investigated 12 week old progeny of mice treated with the chemical mutagen N-ethyl-N-nitrosourea (ENU) using phenotypic assessments that included dysmorphology, radiography, dual-energy X-ray absorptiometry (DEXA). These studies identified a mouse with kyphosis, designated Kylb. Inheritance testing revealed the phenotype to be autosomal recessive trait, Kylb mice, when compared to unaffected littersmates, had a 12% lower body weight (P<0.05); a 7% greater length (P<0.001); a 44% decrease in fat mass (P<0.05); a 14% increase in bone area (P<0.01); and a 6% reduction in areal bone mineral density (P<0.05). The characteristic hunch back deformity was present in mice by 10 days of age. Radiology and whole-skeletal staining using alcin blue and alizarin red revealed Kylb mice to have lumbo-thoracic kyphosis, longer vertebrae and long bones. Genetic mapping localised the Kylb locus to a 5.5 Mb region on chromosome 15 which contains 51 genes, including the natriuretic peptide receptor 3 (Npr3) gene. DNA sequence analysis of Npr3 identified a T to A transversion at codon 209, which altered a highly conserved tyrosine (Tyr) residue to an asparagine (Asn) using phenotypic assays that included dysmorphology, radiography, dual-energy X-ray absorptiometry (DEXA). These studies identified a mouse with kyphosis, designated Kylb. Inheritance testing revealed the phenotype to be autosomal recessive trait, Kylb mice, when compared to unaffected littersmates, had a 12% lower body weight (P<0.05); a 7% greater length (P<0.001); a 44% decrease in fat mass (P<0.05); a 14% increase in bone area (P<0.01); and a 6% reduction in areal bone mineral density (P<0.05). The characteristic hunch back deformity was present in mice by 10 days of age. Radiology and whole-skeletal staining using alcin blue and alizarin red revealed Kylb mice to have lumbo-thoracic kyphosis, longer vertebrae and long bones. Genetic mapping localised the Kylb locus to a 5.5 Mb region on chromosome 15 which contains 51 genes, including the natriuretic peptide receptor 3 (Npr3) gene. DNA sequence analysis of Npr3 identified a T to A transversion at codon 209, which altered a highly conserved tyrosine (Tyr) residue to an asparagine (Asn) residue. Thus, our studies which have established a mouse model for kyphosis due to excessive vertebral growth will help to identify the role of Npr3 in regulating the cellular and molecular mechanisms of vertebral bone growth.

**OC4.2**

**Is Mepe a novel regulator of growth plate mineralisation?**

Katherine Staines, Vicky MacRae & Colin Farquharson Roslin Institute and R(D)SVS, The University of Edinburgh, Roslin, UK.

Advances in the understanding of hypophosphatemic disorders have identified a novel group of molecules (FGF23, PHEX, MEPE, DMP1) that have been implicated in osteoblast mineralisation directly. The specific binding of PHEX to type I collagen (CTX) responses to graded T3-suppression-TRH-stimulation testing in control and RTH subjects. Fasting samples were collected prior to TRH administration and for 6 h. Testing was repeated three times following increasing doses of T3 (50, 100, 200 µg/day for 3 days), and TSH, osteocalcin and CTX were determined in all samples. Baseline TSH increased 5.81±0.29 fold (P<0.0001) in controls and 4.0–6.8 fold (P<0.0001) in RTH patients following TRH-stimulation. TSH responses to TRH were suppressed by T3 in control subjects but not RTH patients. Baseline osteocalcin (P<0.0001) and CTX (P<0.0001) were higher in controls and T3 treatment increased osteocalcin 1.55±0.08 fold (P<0.0001) and CTX 1.37±0.21 fold (P<0.05). In RTH patients, osteocalcin and CTX responses to T3 were impaired and variable, reflecting heterogeneity of the condition and the different THRB mutations identified. By contrast, osteocalcin and CTX levels in all subjects remained unchanged following TRH administration. These data demonstrate that T3 stimulates bone turnover, whereas the rise in TSH following TRH-stimulation does not inhibit osteocalcin or CTX. Thus, rapid bone turnover responses to increased HPT-axis axis activity result from stimulatory actions of thyroid hormones and not the loss of any inhibitory effects of TSH.

**OC4.3**

**Rapid bone turnover responses to increased hypothalamic–pituitary–thyroid–axis activity are mediated by thyroid hormones**

Apostolos Gogakos, Elaine Murphy, Duncan Bassett & Graham Williams

Endocrine Abstracts (2011) Vol 25

Increased hypothalamic–pituitary–thyroid (HPT) axis activity results in high bone turnover. T3 stimulates osteoblast and osteoclast activities, whereas TSH is proposed to inhibit bone turnover directly. Resolving the relative importance of T3 and TSH is complicated by their physiological inverse relationship. We studied 10 controls and 4 patients with resistance to thyroid hormone (RTH), in which mutation of thyroid hormone receptor beta (THRB) disrupts HPT axis negative feedback. RTH is characterized by increased T3, TSH and TSH concentrations and a TSH response to TRH-stimulation that is not suppressed by T3. We hypothesized that skeletal effects of T3 and TSH could be discriminated by comparing bone formation (osteocalcin) and resorption (C-telopeptide of type 1 collagen (CTX)) responses to graded T3-suppression-TRH-stimulation testing in control and RTH subjects. Fasting samples were collected prior to TRH administration and for 6 h. Testing was repeated three times following increasing doses of T3 (50, 100, 200 µg/day for 3 days), and TSH, osteocalcin and CTX were determined in all samples. Baseline TSH increased 5.81±0.29 fold (P<0.0001) in controls and 4.0–6.8 fold (P<0.0001) in RTH patients following TRH-stimulation. TSH responses to TRH were suppressed by T3 in control subjects but not RTH patients. Baseline osteocalcin (P<0.0001) and CTX (P<0.0001) were higher in controls and T3 treatment increased osteocalcin 1.55±0.08 fold (P<0.0001) and CTX 1.37±0.21 fold (P<0.05). In RTH patients, osteocalcin and CTX responses to T3 were impaired and variable, reflecting heterogeneity of the condition and the different THRB mutations identified. By contrast, osteocalcin and CTX levels in all subjects remained unchanged following TRH administration. These data demonstrate that T3 stimulates bone turnover, whereas the rise in TSH following TRH-stimulation does not inhibit osteocalcin or CTX. Thus, rapid bone turnover responses to increased HPT-axis activity result from stimulatory actions of thyroid hormones and not the loss of any inhibitory effects of TSH.

**OC4.4**

**CB1 receptor mediates the effects of glucocorticoids on AMPK activity in the hypothalamus but not in adipose tissues**

Miski Sceif1, Blerina Kola1, Csaba Feke1, Ashley B Grossman1 & Márta Korbonits1

1Department of Endocrinology, Barts and the London Medical School, William Harvey Research Institute, Queen Mary University of London, London, UK; 2Department of Endocrine Neurobiology, Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary.

Introduction

Adenosine monophosphate-activated protein kinase (AMPK) is a regulator of cellular and systemic energy homeostasis. Many of the changes seen in glucocorticoid excess correspond to the metabolic steps regulated by AMPK. In the hypothalamus and adipose tissues, glucocorticoids and cannabinoids share the same tissue specific effects on AMPK activity. Cannabinoids have central orexigenic and peripheral metabolic effects via the cannabinoid receptor type 1 (CB1). The cannabinoid-CB1 system interacts with a number of hormonal systems and perhaps mediates their effects.
Aims
To investigate if the CB1 receptor is required for the tissue-specific effects of glucocorticoids on hypothalamic and adipose tissue AMPK activity.

Methods
Wild-type (WT) and CB1-KO mice were treated for 2 weeks with a surgically implanted pellet containing either cholesterol (control) or corticosterone (5 mg).

Hypothalamic and adipose tissue AMPK activity was determined in a functional kinase assay by the entity of 32P incorporation into the synthetic AMPK substrate SAMS.

Results
Corticosterone significantly increased hypothalamic AMPK activity in WT mice (P<0.0001), but not in CB1-KO mice (P=0.636). WT mice treated with corticosterone, as compared to cholesterol treated controls, showed significantly decreased visceral (mesenteric) and subcutaneous (inguinal) fat AMPK activity (P<0.001 for both). Corticosterone treated CB1-KO mice also showed significantly decreased visceral and subcutaneous fat AMPK activity as compared to cholesterol-treated controls (P=0.0017 and P=0.0013, respectively). However, CB1-KO mice showed higher baseline visceral (P=0.0125) and subcutaneous (P=0.0242) adipose tissue AMPK activity as compared to WT mice.

Conclusion
The stimulatory effects of glucocorticoids on hypothalamic AMPK activity are CB1-dependent. The CB1 receptor does not mediate the inhibitory effect of glucocorticoids on adipose tissue AMPK activity but has an independent inhibitory effect on adipose tissue AMPK activity.

Endocrine Abstracts (2011) Vol 25

OC4.5
Alterations to hypothalamic 5-HT and DA turnover in offspring induced by maternal exposure to a high caloric diet throughout lactation

Thomas Weight1, Peter Voigt2 & Simon Langley-Evans2
1School of Veterinary Medicine and Science, University of Nottingham, Loughborough, Lecestershire, UK; 2School of Biosciences, University of Nottingham, Loughborough, Lecestershire, UK.

Exposure to maternal obesity or overfeeding during early development has lasting effects upon the young adult rat. Maternal cafeteria (CD) feeding during lactation programmes behaviour in the adult offspring, reducing anxiety in males and altering the behavioural satiety sequence in females. The aim of the present study was to examine the impact of early exposure to maternal over-nutrition upon bioactive amines in the brain. Lactating Wistar rats were fed either a control chow (n=5) or CD (n=5). CD consisted of a range of highly palatable human food items, high in fat and sugar (including cheese, pate, pork-pie, peanut, chocolate, crisps and shortbread). At weaning all offspring were transferred to the control diet. At 20 weeks of age offspring were culled and the hypothalamus, hippocampus and frontal cortex were dissected. 5-HT, 5-HIAA, DA, DOPAC and HVA concentrations were measured using HPLC. Exposure to CD during the suckling period had little effect upon body weight and adiposity of the offspring, although CD females had 39% more perirenal fat than controls (P<0.001). Hypothalamic concentrations of 5-HT were increased by 33% in both male and female CD offspring (P<0.001). In these animals, 5-HT turnover was significantly reduced (37%) in the hypothalamus (P<0.0001). In CD females, DA concentrations were increased (37%, P<0.05) and DOPAC concentrations were reduced (38%, P<0.05) in the hypothalamus. Hypothalamic DA turnover was significantly decreased in both male (31%, P<0.01) and female (22%, P<0.01) CD offspring. There was no effect of maternal diet upon 5-HT or DA turnover in the hippocampus or frontal cortex. The present findings suggest early life programming of hypothalamic 5-HT and DA turnover in response to maternal over-feeding. The data is suggestive of a role for these neurotransmitters in determining the altered behaviour of such animals.

OC4.6
Dual effect of arachidonic acid on peroxisome proliferator-activated receptor γ (PPARγ)-dependent action in 3T3-L1 adipocytes
Emma D Nikolopoulou, Malcolm Parker & Mark Christian
Imperial College London, London, UK.

Dietary fat has been correlated with obesity since it induces the proliferation and differentiation of pre-adipocytes. Now it has become clear that the effect of fat on human health depends on the composition and the nature of fatty acids. Arachidonic acid (AA) is a major omega-6 polyunsaturated fatty acid (PUFA) with a controversial role in adipocyte differentiation. We investigated the effect on pre-adipocyte differentiation following a brief exposure to AA.

We show that a short treatment of 3T3-L1 cells with AA at the start of differentiation induces events with a long lasting inhibitory effect on adipogenesis. Treatment of pre-adipocytes with AA for only 24 h prevented differentiation since the expression of adipocyte markers (PPARγ, cEBPβ etc.) and lipid accumulation were significantly reduced after 10 days of differentiation. In addition, after 24 h of differentiation in the presence of AA treatment the fatty acid binding protein 4 (FABP4 or ap2) expression was induced 100-fold. We show that this effect is PPARγ-dependent since AA treatment was unable to induce ap2 upregulation in PPARγ knockdown cells. This appears to be a gene-specific effect as other PPARγ targets were not affected by AA. Treatment with indomethacin, a general cyclooxygenase inhibitor, blocked AA action on ap2 expression indicating that this effect is prostaglandin-mediated.

We suggest that a short treatment with AA during the early stages of adipocyte differentiation regulates PPARγ expression and/or activity with two very different outcomes. On the one hand, PPARγ in the presence of AA causes the rapid early upregulation of its primary target ap2. On the other hand, at the later stages of differentiation in the absence of AA, PPARγ expression fails to be induced and initiate the differentiation program.
OC4.8
Hyperghrelinaemia, hyperphagia, food hoarding and reduced adiposity in an imprinting centre deletion mouse model of Prader–Willi syndrome
Timothy Wells1, Đinko Relkovic2, Hannah Furby1, Irina Guschina1, Sachiko Nishimura1, James Resnick2 & Anthony Isles2
1School of Biosciences, Cardiff University, Cardiff, UK; 2Schools of Medicine and Psychology, Cardiff University, Cardiff, UK; 3School of Optometry, Cardiff University, Cardiff, UK. 4Center for Mammalian Genetics, University of Florida, Gainesville, Florida, USA.

Prader–Willi syndrome (PWS) is a neurodevelopmental disorder caused by a lack of paternal gene expression from 15q11–q13 and is characterised by failure to thrive in infancy, followed by hyperphagia due to abnormal satiety responses and increased motivation by food. We investigated growth and metabolism a mouse model in which the imprinting centre (IC) of the homologous PWS interval has been deleted (PWS-IC mice). Growth retardation only emerged post-natally, with adult PWS-IC mice weighing 40% less than controls, and tibia length reduced by 7%. In contrast, liver and brain weights were normal. Analysis of daily food intake over a 3-week period indicated that male PWS-IC mice showed proportionate hyperphagia, which became more pronounced after overnight fasting. In addition, PWS-IC mice displayed ‘food hoarding’ behaviour reminiscent of that seen in PWS individuals. This hyperphagia may result from the 3-fold elevation in circulating ghrelin levels. Despite hyperghrelinaemia and hyperghrelinaemia, PWS-IC mice were remarkably lean, with proportionate retroperitoneal, epididymal and inguinal white adipose tissue weights reduced by 82, 84 and 67% respectively, and proportionate interscapular brown adipose tissue weight reduced by 48%, with additional reductions in marrow adiposity and hepatic lipid content. This lack of body fat may explain the 1.3°C reduction in surface body temperature in PWS-IC mice, consistent with reduced heat retention. In addition, despite proportionate over-eating, and in contrast to wild-type controls, PWS-IC mice did not gain fat mass, even when fed on a high-fat diet. Our data show that the neuroendocrine regulation of metabolism is severely compromised in PWS-IC mice and that loss of paternal gene expression from the PWS-cluster gives rise to hyperphagia, and hyperghrelinaemia, as seen in the human condition. However, unlike individuals with PWS, the PWS-IC mice do not become obese. Whether this difference is due to the elevated energy demands of increased heat loss remains unclear.

OC5.2
Maternal low protein diet and fetal growth restriction: new insights into the role of placental 11β-hydroxysteroid dehydrogenase-2
Elizabeth Cottrell, Megan Holmes & Jonathan Seckl
University of Edinburgh, Edinburgh, UK.

Placental 11β-hydroxysteroid dehydrogenase-2 (11β-HSD2) rapidly converts glucocorticoids to inactive metabolites, thus protecting the developing fetus from high maternal glucocorticoids. Genetic deficiency or inhibition of 11β-HSD2 associates with fetal growth restriction, low birth weight, and cardiometabolic disease in adulthood. Similar ‘programming’ effects are seen with maternal malnutrition or stress; these challenges associate with reduced placental 11β-HSD2 at term. We therefore explored the importance of 11β-HSD2 in dietary programming.

Feeding C57Bl/6J mice an isocaloric low protein (LP) diet throughout gestation (8% protein versus 18% control diet) significantly decreased placental weight ($P<0.001$) and fetal weight at embryonic day (E) 17.5 (1.75 (0.98 ± 0.05) g in control versus 0.74 ± 0.03 g in low protein; $P<0.001$). This reduction in fetal weight was preceded by a downregulation of 11β-HSD2 enzyme activity from E16.5 (decreased in LP by 28 and 20% at E16.5 and 17.5, respectively; $P<0.05$). However, at earlier gestational ages (E13.5–15.5), LP diet increased placental 11β-HSD2 activity (increased in LP by 21, 23 and 41% at E13.5, 14.5 and 15.5, respectively; $P<0.05$), perhaps to maximise mdestation fetal growth. Using a heterozygous 11β-HSD2 +/- mating strategy to generate 11β-HSD2 +/- and +/- +/- offspring within the same litters, maternal LP diet reduced fetal body weight to a similar extent in all genotypes compared with control offspring, although the magnitude was blunted in 11β-HSD2 +/- offspring. Thus, LP diet has effects over-and-above 11β-HSD2 inhibition to reduce fetal weight gain.

OC5.3
Mutations in the gene encoding the fibroblast growth factor factor 8 (FGF8) are associated with complex midline defects including recessive holoprosencephaly and hypothyphalmo-pituitary dysfunction
Mark McCabe1, Carles Gaston-Massuet2, Vaira Tziarfi1, Louise Gregory1, Kyriaki Alatzoglou1, Massimo Signore2, Suda Farooq1, Jamal Raiza1, Joanna Walker1, Scott Kavage3, Pei-San Tsai4, Nelly Pitteloud5, Juan-Pedro Martinez-Barrera6 & Mehrul Dattani1
1Clinical and Molecular Genetics Unit, Institute of Child Health, London, UK; 2Harvard Reproductive Endocrine Sciences Center and Reproductive Endocrine Unit of the Department of Medicine, Boston, Massachusetts, USA.

Loss-of-function mutations in FGF8 in humans have been associated with Kallmann syndrome (KS), which is characterised by the combination of hypogonadotrophic hypogonadism with anosmia, suggesting that FGF8 is critical for GnRH neuronal development. Interestingly, hypomorphic FGF8 mutant mice demonstrate poor telencephalic development with deletions of midbrain tissue, absence of olfactory bulbs and optic chiasm, and holoprosencephaly (HPE) with an abnormal corpus callosum; thus it appears that FGF8 is important for forebrain development, but its role in hypothyphalmo-pituitary (HP) development remains to be determined. We aimed to investigate the role of FGF8 in the formation of midline forebrain, HP and craniofacial development in mice and human.

Patients with congenital hypothyphuitarism and midline forebrain/craniofacial defects (n=421) were screened for FGF8 mutations. Two novel missense mutations were identified: i) homozygous p.R189H and ii) heterozygous p.Q216E. The p.Q216E mutation was identified in a female patient with an absent corpus callosum, hypoplastic optic nerves and Moebius syndrome. In situ hybridisation revealed FGF8 expression in human embryonic ventral diencephalon and anterior forebrain but not in Rathke’s pouch, which parallels known patterning in mice. Additionally, hypomorphic mice showed a reduction in

Endocrine Abstracts (2011) Vol 25

Society for Endocrinology BES 2011, Birmingham, UK
vasopressin and oxytocin, with small/hyperplastic anterior pituitary glands and absent posterior pituitary glands in the most severely affected mice. To conclude, we demonstrate the first recessive case of HPE associated with a mutation in FGF8, a condition previously associated with heterozygous mutations in SDX3, TGFβ and the Sonic Hedgehog signalling pathway. We have shown that Fgf8/Fgf8 appears to be important for forebrain and HP development in mouse and human, with overlapping phenotypes between KS and complex midline defects with hypopituitarism. Furthermore, this is the first report to implicate FGF8 mutations in Moebius syndrome.

**OCS.4**

Impaired cardiac function in GR<sup>−/−</sup> fetal mice

Eva Rog-Zielinska<sup>1</sup>, Adrian Thomson<sup>1</sup>, Carmel Moran<sup>1</sup>, David Brownstein<sup>1</sup>, Christopher Kenyon<sup>2</sup>, Zoi Michailidou<sup>1</sup>, Dorota Szumskaz<sup>1</sup>, Shoumo Bhattacharya<sup>1</sup>, Jennifer Richardson<sup>1</sup>, Elizabeth Owen<sup>1</sup>, Alistair Watt<sup>1</sup>, Lesley Forrester<sup>1</sup>, Megan Holmes<sup>1</sup> & Karen Chapman<sup>1</sup>

<sup>1</sup>Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK; <sup>2</sup>Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, UK.

Glucocorticoid levels rise dramatically in late gestation in mammals and are essential for organ maturation in preparation for birth. They are widely used clinically to mature the lungs of premature infants and hypomorph GR mice die neonatally due to severe lung atelectasis. Here, we describe fetal lethality in GR null (GR<sup>−/−</sup>) mice with 51% loss at E17.5 (P<0.05) that could be due to impaired cardiac development. Histology showed reduced heart size in GR<sup>−/−</sup> fetuses from E16.5, co-incident with a peak in heart corticosterone content, which was undetectable at E14.5. Magnetic resonance imaging confirmed a 21.9 ± 4.7% reduction in ventricular volume at E17.5 (P<0.05) yet no structural abnormalities were observed. In vivo high frequency ultrasound analysis revealed impaired left ventricular function in E17.5 GR<sup>−/−</sup> fetuses, with increased TEI-Doppler index (WT, 0.36 ± 0.02 versus KO, 0.54 ± 0.03, P<0.001), with heterozygous mice showing an intermediate phenotype (0.45 ± 0.02; P<0.01 versus WT), and decreased E/A wave ratio (WT, 0.41 ± 0.028 versus KO, 0.28 ± 0.038). Impaired cardiac performance was accompanied by oedema in GR<sup>−/−</sup> fetuses (wet weight WT, 0.85 ± 0.03 versus KO, 0.86 ± 0.01, dry weight WT, 0.12 ± 0.04 versus KO, 0.1 ± 0.02, P<0.01; fetal Na/K ratio WT, 0.9 ± 0.02 versus KO, 1.03 ± 0.01, P<0.001), indicative of heart failure. The observed phenotype could not be attributed to deficiency in cardiac adrenaline content, which was normal at E17.5, nor were there any compensatory alterations in cardiac mineralocorticoid receptor mRNAs levels. Cardiomyocytes of E17.5 GR<sup>−/−</sup> hearts displayed cellular defects, including undifferentiated myofibrils and dense nuclei. RNA analysis showed normal cardiac gene expression in GR<sup>−/−</sup> fetuses at E14.5 (prior to the glucocorticoid increase), but a lack of the normal maturational changes at E17.5 (e.g. increased α-myosin heavy chain, increased PC1α, decreased UCP2). These data suggest that glucocorticoid action in late gestation heart is essential for its biochemical and functional maturation and lack of the normal maturational effects of glucocorticoids leads to congestive heart failure.

This work was supported by a British Heart Foundation studentship (to E R-Z) FS08/065.

**OCS.5**

Absence of 11β-HSD2 specifically within the fetal brain alters adult 'depressive' behaviour

Caitlin Wyrwoll, Jonathan Seckl & Megan Holmes

The University of Edinburgh, Edinburgh, UK.

Prenatal glucocorticoid overexposure is a key risk factor for susceptibility to neuropsychiatric disorders in adult life. Fetal exposure to glucocorticoids is regulated by 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) an enzyme which inactivates glucocorticoids and is highly expressed in the placenta and fetus. Previous work has established that 11β-HSD2<sup>−/−</sup> offspring generated by heterozygous matings exhibit altered placental development and function, decreased birth weight, delayed neurodevelopment and increased anxiety and depression as adults. This raises the question as to whether it is placental or fetal 11β-HSD2 that are key to subsequent programmed outcomes. The current study investigated the significance of neural 11β-HSD2 on adult behaviour. A knockout of 11β-HSD2 specifically within the brain during development was created by crossing NestinCre mice with floxed 11β-HSD2 mice (HSD2<sup>flx/flx</sup>). 11β-HSD2 activity in fetal tissue and placenta was measured at E12.5, neurodevelopmental landmarks assessed and adult behaviour characterised. Brain-specific reduction in 11β-HSD2 activity was confirmed at E12.5 in the fetal heads of Nestin-Cre.HSD2<sup>−/−</sup> mice in comparison to HSD2<sup>flx/flx</sup> (2.01 ± 0.24 and 10.13 ± 1.69 mU/mL, P<0.001). Birth weight and negative geotaxis and eye opening, markers of neurodevelopment, were unaltered. Anxiety was assessed by elevated plus maze and open field in the adult offspring and was unchanged between the two genotypes. However depressive-like behaviour, as assessed by the tail suspension test, was increased in mice with brain-specific deletion of 11β-HSD2 with NestinCre.HSD2<sup>flx/flx</sup> spending a greater percentage of time hanging in comparison to the HSD2<sup>flx/flx</sup> mice (68 and 53% respectively, P<0.05). Our data suggest that neural 11β-HSD2 does indeed have a role in protecting the developing brain and thus determining adult psychiatric pathology. However, to prove to be a minor role compared to that of 11β-HSD2 in the placenta as the current observed phenotype appears more subtle than the global 11β-HSD2 knockout.

**OCS.6**

Consequence of embryonic exposure to 17β-estradiol on brain asymmetry development and neuroendocrine gene expression in Danio rerio

Madeleine Pope, Julien Lambertucci-Bonnett, Imelda McGonnell, Robert Fowkes & Claire Russell

Royal Veterinary College, London, UK.

Exposure to a range of environmental endocrine disrupting chemicals is associated with birth defects in several species. High levels of oestrogen, or compounds acting as oestrogen mimics (such as alkyl phenols and pesticides) can alter gene expression and cause sex reversal in fish. In the current study, we use the versatile Zebrafish (Danio rerio) to examine the effect of embryonic exposure to 17β-estradiol (E<sub>2</sub>) on the development of brain asymmetry and neuroendocrine gene expression. Brain asymmetry was monitored using an assay of parapineal migration, which employs transgenic zebrafish (Tg(foxD3:GFP)) in which the pinel complex is labeled with GFP. The effect of E<sub>2</sub> on the hypothalamus and pituitary was assayed using wholemount in situ hybridization (ISH) of previously validated pome and pmr mRNA probes. Embryos were exposed to E<sub>2</sub> in aquaria water (0, 100 nM, 1 μM, 10 μM) from for 3 days at 28 °C. At 72 hpf, the embryos were viewed under an microscope equipped to visualise GFP and scored according to the appearance of the parapineal organ (left-handed, right-handed or no migration). Embryos were fixed in 4% paraformaldehyde prior to ISH. Initial results of the parapineal migration assay suggest a decreased incidence of rightward migration of the parapineal organ in embryos exposed to E<sub>2</sub> for 72 hpf. There was also an increased incidence of parapineal organ migration failure, suggesting inappropriate exposure to E<sub>2</sub> during development could cause migrational defects associated with neurological disorders (e.g. autism, schizophrenia). Neuroendocrine gene expression studies revealed enhanced pmr expression in the pituitary of E<sub>2</sub>-treated embryos, although it was unclear as to whether this represented increased gene expression or lactotroph hyperplasia. Furthermore, the spatial profile of hypothalamic and pituitary pome expression was also altered by E<sub>2</sub> exposure. Collectively, these data suggest that neurological and neuroendocrine changes occur following a short exposure to E<sub>2</sub>, which may result in abnormal development.

**OCS.7**

Plasma biomarkers for early prediction of preeclampsia

David Carty, Lesley Anderson, Jim Mcculloch, Anna Dominiczak & Christian Delles

Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK.

Introduction

Several biomarkers are elevated in late pregnancy in women with preeclampsia (PE), but none have been sensitive or specific enough to be used routinely in early pregnancy to predict the condition. We measured plasma levels of the anti-angiogenic peptides FMS-like tyrosine kinase 1 (sFLT-1), soluble endoglin (sENG), placental growth-factor (PGF), and an array of cytokines and adhesion molecules in early and mid pregnancy to assess their ability to predict PE.

Methods

Two thousand women were recruited at gestational week 12–16 (booking); 36 (1.9%) developed PE, and were matched for age and BMI to 74 women who had non-preeclamptic pregnancies. Fifty-two women with 2:2 risk factors were also recruited at gestational week 28, of whom 11 developed pre-eclampsia. sFLT-1, sENG and PGF levels were measured using ELISA kits (R&D Systems); levels of cytokines

Endocrine Abstracts (2011) Vol 25
Results
Systolic blood pressure was not different, but diastolic blood pressure higher (74 ± 10 vs 69 ± 9 mmHg; \( P = 0.022 \)) at booking in women who developed PET. sENG was higher (6.96 ± 3 vs 5.72 ± 2.1 ng/ml, \( P = 0.025 \)), and PGF lower (25.8 ± 27 vs 37.4 ± 30 pg/ml, \( P < 0.001 \)) at booking in affected women. sFLt-1 was not different between the 2 groups at booking (\( P = 0.52 \)), but higher in affected women at week 28 (1979 ± 1118 vs 1230 ± 624 pg/ml, \( P = 0.009 \)). Of the cytokines and growth factors, E-Selectin was higher in women who developed PET, both at booking (15.1 ± 4.9 vs 12.9 ± 4.5 ng/ml, \( P = 0.022 \)) and week 28 (14.4 ± 5.6 vs 10.73 ± 3.5 ng/ml, \( P = 0.01 \)). There was no difference between the 2 groups at either timepoint in levels of VCAM-1, ICAM-1, P-Selectin, L-Selectin, Interleukins 1-α, 1-β, 2, 4, 6, 8 or 10, VEGF, TNF-α, interferon-γ, MCP-1 and EGF.

Conclusion
When measured in early pregnancy, along with maternal risk factors, sENG, PGF and E-Selectin may provide additional prognostic information about future risk of pre-eclampsia.

Q05.8
Altered fetoplacental growth in monocarboxylate transporter 8 (Mct8) knockout mice
Elisavet Vasilopoulou1, Heike Heuer2, Marija Trajkovic2, Laurence Loubiere1, Christopher McCabe1, Jayne Franklyn1, Mark Kilby1 & Shiao Chan1
1University of Birmingham, Birmingham, UK; 2Leibniz Institute for Age Research-Fritz Lipmann Institute, Jena, Germany.

The plasma membrane thyroid hormone (TH) transporter, MCT8, is present in the human placenta from early gestation and is postulated to participate in transplacental transfer of TH. \textit{In vitro}, MCT8 overexpression decreases the survival of human cytotrophoblast in a TH-independent manner.

Objective
To examine the role of Mct8 in fetoplacental growth using the Mct8 knockout (ko) mouse model.

Methods
Heterozygous females were mated with male ko mice. Male wild-type (wt) and ko fetoplastic tissues were obtained from two litters before (E14.5) and after (E18.5) the onset of fetal TH production. Mct8 protein was localized in the mouse placenta by immunohistochemistry. The volume fractions of the labyrinthine (Lz) and junctional (Jz) zones of the placenta were estimated using stereology. Cyclin D1 (proliferation) and Caspase 3 (apoptosis) protein expression was assessed by western immunoblotting.

Results
At both E14.5 and E18.5, Mct8 protein was expressed in decidua, in spongiotrophoblast and glycogen cells in the Jz, in cytotrophoblast and syncytiotrophoblast cells in the Lz, in the wall of chorionic blood vessels and in the chorionic membrane. At E18.5, male ko fetuses were lighter (1273 ± 24.5 mg) than male wt (1397 ± 28.6 mg; \( P < 0.05 \)), whilst their placentae were heavier (ko: 90.3 ± 6.5 mg; wt: 77.3 ± 2.8 mg; \( P < 0.05 \)). This was accompanied by increased Cyclin D1 and decreased Caspase 3 protein expression in ko compared with wt placentae. Fetoplacental weight ratios were decreased (30%; \( P < 0.01 \)) in ko compared with wt placentae with no difference in the absolute volume of Lz.

Conclusions
The ubiquitous localization of Mct8 in mouse placentae is similar to that observed in humans. Its localisation in trophoblasts within the Lz, suggests a role for Mct8 in transplacental transport. Furthermore, lack of Mct8 results in reduced placental efficiency with a compensatory increase in placental size.
Poster Presentations
Bone

P1

Does preoperative localisation for total parathyroidectomy in patients with renal failure improve outcome?

Thomas Hanna, Jo Edwards, Helen Grimsmo & Jacob Akoh
Derriford Hospital, Plymouth, UK.

Background
Secondary hyperparathyroidism is a common complication of established renal failure (ERF) and is associated with significant morbidity and mortality. The aims of this study were to determine patient and operative characteristics, which might predict persistent or recurrent hyperparathyroidism after surgery. To assess the influence of pre-operative imaging on the ability to locate and remove parathyroid glands during both the initial and repeat surgery and to assess the long-term effect of failed surgery.

Methods
A retrospective study of all chronic kidney disease patients who required a total parathyroidectomy because of failed medical management from 1st January 1999 to 31st December 2008. Patient characteristics, preoperative imaging, medical treatment, operative findings, histology and patient outcome were all studied. Differences between groups (dialysis dependent and non dialysis dependent) were tested by the χ2 statistic and a P value of <0.05 was regarded as significant.

Results
Seventy-five patients underwent total parathyroidectomy during this period and were followed up for an average of 44.5 months. Sixty-one (81%) had removal of all parathyroid glands with associated fall in parathyroid hormone level. Pre operative imaging was used in 15 patients (20%) and found to be unhelpful in directing surgery in 12 of 15 (80%) cases. Four patients underwent repeat parathyroid surgery for recurrent/persistent PHPT with pre operative imaging used in two cases.

Conclusion
A high success rate can be achieved without the use of pre-operative imaging and is therefore not indicated prior to the first parathyroidectomy operation. The long-term effects of pharmaceutical developments in this area are, at present unknown but are likely to change indications for initial surgery and reoperation. An agreed protocol is therefore essential for the management of CKD patients with secondary hyperparathyroidism.

P2

Influence of age and gender on expression of primary hyperparathyroidism

Viral Shah, Sanjay Bhadada, Anil Bhansali, Pinaki Dutta & A Behara
Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Background
The geographical difference in presentation of primary hyperparathyroidism (PHPT) is well known; however, there is sparse literature on influence of age and gender on presentation of PHPT.

Objective
To study the effect of age and gender on clinical and biochemical presentation of PHPT.

Method
This is a retrospective analysis of 184 histopathologically proven PHPT patients between March 1990 and March 2010 from a single centre of North India. PHPT patients were divided in five different age groups, i.e. children <12 years, adolescent 12–18 years, young adult 19–25 years, adult 26–59 years and older ≥60 years. Clinical presentations, biochemical parameters and parathyroid gland weight were compared among different age groups and between genders.

Results
Female: male ratio was 70:30. 77.2% were between 25 and 59 years while 5.97% were <12 years. There was no change in clinical presentations and biochemical parameters among the different age groups except rickets in children and adolescent (2.2%), and renal stones in 25–59 years adults (41.3%) and markedly elevated alkaline phosphatase in children and adolescent (P<0.01). Bone pain and muscle aches (P<0.01), fracture (P=0.04), renal stone (P=0.05), cholelithiasis (P=0.03) and pancreatitis (P=0.02) were more common among women. Serum calcium and phosphate levels were similar in either sex but parathyroid hormone (PTH) level was higher among women (P=0.02). Parathyroid adenoma weight was higher in older compare to young but did not reach to level of statistical significance.

Conclusion
There was no much difference in the clinical and biochemical presentation of PHPT among different age groups but differences among genders were noticeable.

P3

Evaluation of the association between serotonin and bone mineral density in patients with neuroendocrine tumours

Pyia Sen Gupta, William M Drake, Scott A Akker, Shern L Chew, Ashley B Grossman & Maralyn R Druce
Barts and the London School of Medicine, QMUL, London, UK.

Introduction
Bone mineral density (BMD) and fracture tendency are influenced by diet, activity, drugs, and hormones. Recent studies highlight an inverse relationship between serotonin and BMD, of uncertain mechanism.

Purpose
We investigated the relationship between serotonin metabolites and BMD in patients with sporadic neuroendocrine tumours (NETs), with and without the carcinoid syndrome.

Materials and methods
One-year prospective audit [ref 09/104]. All patients underwent DEXA-scanning as standard care for chronic malignancy. Data collection included clinical details, confounders influencing BMD and relevant investigation results.

Results
Of 41 eligible patients, 15 did not participate (too unwell, default follow-up, declined participation) and 3 were excluded due to confounding factors. Twenty-three subjects were reviewed (15 females, 59.±9 ±19 years, BMI 26.8 ±5.6, 8 males, 69±±3.0 years BMI 30.6 ±5.6). The mean interval since diagnosis was 5.7 years (range 0–20 years); 16 had carcinoid syndrome, 7 did not. Thirteen had previous surgery, 4 chemotherapy, 11 radiolabelled-MIBG and 14 used somatostatin analogues. Low BMD was not prominent: only 4 subjects had Z score >1 s.d. below the mean, with no unifying features in this group, 10 subjects had T score ≤−1.0 (osteopenia or osteoporosis), all were females (P<0.01), and 7 had the carcinoid syndrome (P>0.05). Mean BMD was 25.4 cf 30.2 for those with normal T score (P<0.05). The low T score group did not differ from the group with a normal T score in age, smoking, alcohol, medications, calcium or TSH but mean vitamin D was higher (77.8 vs 48.6 nmol/l; P=0.05). There was no difference in chromogranin-A (217.9 vs 281.9 nmol/l; P=0.6) or urine 5HIAA (360.8 vs 147.5 µmol/24 h; P=0.3). Z scores did not differ between subjects with elevated or normal 5HIAA (P=0.28) or between subjects with and without the carcinoid syndrome (P=0.38).

Conclusion
NET patients do not show lower BMD related to serotonin metabolites or disease markers, although a larger cohort is required for confirmation of these preliminary data.

P4

Management of hypovitaminosis D in patients with primary hyperparathyroidism

Manjusha Rathi, Susana Gonzalez & Steve Peacey
Bradford Teaching Hospitals NHS Trust, Bradford, UK.

Epidemiological studies suggest that hypovitaminosis D is common in patients with primary hyperparathyroidism (PHPT). They have higher levels of serum parathyroid hormone (PTH) and markers of bone turnover, and more frequent fractures than vitamin D replete patients. There are concerns that vitamin D repletion in these patients might exacerbate pre-existing hypercalcaemia, although recent literature suggests this is uncommon.

We aimed to determine the effects of vitamin D replacement on biochemical indices of calcium metabolism in patients with combined PHPT and hypovitaminosis D in a predominantly Asian cohort.

Twenty-three patients with PHPT and hypovitaminosis D were studied: Asian/Caucasian 19:4; M:F 2:1; age (range) 59 (31–85) years; basal calcium (mean ± s.d.) 2.62 ±0.12 mmol/l, 25-OH vitamin D 14.8 ±7 nmol/l. Each received oral 20 000 IU cholecalciferol per week for 12 weeks.

Mean baseline serum calcium, phosphate, alkaline phosphatase and PTH were measured at week 4, 8 and 12 weeks. Mean 25-OH vitamin D levels before and after 12 weeks were compared and the relationship between 25-OH vitamin D and PTH was analysed.

A significant increase in 25-OH vitamin D was observed at 12 weeks: 14.8 ±7 vs 75.8 ±21 nmol/l, P=0.001. A reduction in PTH was found at week 8; 21.9 ± 11 vs 15.3 ± 6, P=0.05, and at week 12; 21.9 ± 11 vs 14.7 ± 5, P=0.02. There was no significant change in calcium (P=0.85), phosphate (P=0.37) or alkaline phosphatase (P=0.94). PTH and 25-OH vitamin D levels were inversely correlated (r = −0.46; P=0.002).

Conclusion
We conclude that weekly vitamin D supplementation with 20 000 IU for a three month period corrects hypovitaminosis D in patients with mild PHPT without causing significant increases in calcium.
P5 Risk factors for femoral neck fracture in Indian postmenopausal women (BMD, Vitamin D study and Propensity for a fall)
Thomas Paul, Sivan Arulselvan, Nihal Thomas, Mandalam Seshadri & Arun Jose
Christian Medical College, Vellore, India.

Introduction
Hip fractures are the most serious osteoporotic fractures and difficult to prevent without a precise knowledge of the causative factors. The risk factors, which precipitate hip fracture in elderly may vary according to the local customs and practices. Indian data on risk factors for hip fractures are scant. The present study was undertaken to assess the various risk factors leading to femoral neck fracture in postmenopausal women and also to define the threshold for BMD at femoral neck below which the risk for fracture is increased.

Methods
This study included 31 postmenopausal subjects with femoral neck fracture and 31 age and BMI matched controls. Drug history, history of other systemic illnesses and visual and hearing impairment were assessed. Serum alkaline phosphatase, albumin, creatinine and 25 (OH) vitamin D and intact PTH were also assessed. Bone mineral density was assessed by using the DXA scanner at the lumbar spine and the femoral neck.

Results
Sixtyseven more patients with femoral neck fracture were using sedatives and had hearing and visual impairment compared to controls (P<0.05). Mean serum albumin, serum 25(OH) vitamin D and femoral neck BMD were significantly lower in femoral neck fracture group. Seventy four percent in the fracture group had vitamin D deficiency (<20 ng/ml) when compared to 45% in the control group (P=0.05).

Conclusion
Significant proportion of femoral neck fracture patients had visual and hearing impairment and was using sedatins. They also had vitamin D deficiency. These factors are correctable and need special attention in the Indian context.

P6 Effect of age and gender on bone turnover markers: relationships with oestradiol and parathyroid hormone
Miguel Debono, Fatima Gissiell, Jennifer Walsh & Richard Eastell
Academic Unit of Bone Metabolism, University of Sheffield, Sheffield, UK.

Aims/hypothesis
Bone turnover markers mainly reflect three processes: bone remodelling, linear growth, and bone modelling. These occur at different rates depending on age. Oestradiol levels, a mediator of all processes of bone turnover, vary according to sex and age whereas, parathyroid hormone levels, an important regulator of bone remodelling, vary with age. We hypothesised that gender and age differences in the bone formation marker, serum amino terminal propeptide of type 1 collagen (PINP) and the bone resorption marker, serum b carboxy (C) terminal telopeptide (bCTX), could be related to differences in these hormones.

Methods
We performed an observational, cross-sectional study in 180 participants, 90 men and 90 women, divided into three groups (30/group) by age: Group A 16–18 years, Group B 30–32 years and Group C over 70 years. In all individuals we measured fasting serum oestradiol, parathyroid hormone, serum bCTX and PINP at 9am.

Results
In Group A (16–18 years) serum bCTX (P<0.001); PINP (P<0.001) and in Group B (30–32 years) serum bCTX (P<0.001); PINP (P=0.003) were significantly higher in males compared to females whilst in the elderly Group C values were similar: serum bCTX (P=0.2) PINP (P=0.2). Using multiple linear regression age, sex and oestradiol were significant predictors for serum bCTX (P<0.001), (P=0.006) and PINP (P<0.001); (P=0.00) and (P=0.02), respectively. PTH had no effect whilst oestradiol was a negative predictor for bone marker levels.

Conclusions/interpretation
Younger men were found to have higher bone turnover markers then women but levels were similar in the elderly. This study highlights the importance of having different bone turnover marker reference intervals for young men and women.

P7 Vitamin D insufficiency in hyperparathyroidism
Myint M Aye1, Mo Aye1, T Sathyapalan1, E S Kilpatrick & Stephen L Akin1
1University of Hull, Hull, UK; 2Hull and East Yorkshire Hospitals NHS Trust, Hull, UK.

Background
Vitamin D insufficiency is very common in general population and in patients with primary hyperparathyroidism (PHPT). Measurement of serum 25OHD (25hydroxy-vitamin D) is currently the best available test to assess vitamin D adequacy. However, it has a significant seasonal variation, and is difficult to define a serum 25OHD level that is sufficient for bone health, although a serum 25OHD above 50 nmol/l has been suggested. Previous studies confirm a significant negative correlation between 25OHD, and parathyroid hormone (PTH), alkaline phosphatase that is reversible. In the NHANES study the higher serum 25OHD then the higher the BMD throughout the reference range. The greatest impact of vitamin D on PTH is found when 25OHD is <50 nmol/l. PTH levels have been shown to decline until 25OHD above 75 nmol/l.

Aim
To determine if a single recorded vitamin D level above 50 nmol/l in routine clinic practice can safely exclude vitamin D deficient secondary hyperparathyroidism.

Study method
A retrospective study of 480 postmenopausal women who had high PTH levels during screening at an osteoporosis clinic was conducted.

Results
Fifty-two of 480 patients (10.8%) had PHPT, 19 (4%) had renal related secondary hyperparathyroidism, and 222 (46.25%) had 25OHD level <50 nmol/l. The remaining 170 who had normal renal function, normal adjusted calcium but 25OHD >50 nmol/l and a high PTH appeared to have normocalcaemic hyperparathyroidism. Six month later, with continued replacement of vitamin D and calcium, PTH was normalized in 65 patients, remained high with normal calcium in 55 patients and not checked in 47 patients. Three progressed to hypercalcaemic PHPT. 38 of 55 patients who remained normocalcaemic hyperparathyroidism showed 25OHD <50 nmol/l on repeat testing.

Conclusion
The 25OHD level is variable and a single measurement may not exclude vitamin D insufficiency. The observation also suggested that many cases of normocalcaemic hyperparathyroidism might simply have or coexist with vitamin D insufficiency.

P8 Thyrotratin expression and signalling in the skeleton
J H Duncan Bassett1, Rebecca Hernandez2, Charlotte Combs3, Anne van der Spek1, Ming Yu1, Allan Williams1, Elaine Murphy1, Alan Boyde2, Clementine J J van Zeijl3, Anita Boelen3 & Graham R Williams1
1Molecular Endocrinology Group, Department of Medicine and Medical Research Council Clinical Sciences Centre, Imperial College London, Hammersmith Hospital, London W12 0NN, UK; 2Oral Growth and Development, Institute of Dentistry, Bart’s and the London School of Medicine, Queen Mary, University of London, London E1 1BB, UK; 3Department of Endocrinology and Metabolism, Academic Medical Center, University of Amsterdam, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

Hypothyroidism and thyrotoxicosis have detrimental effects on skeletal development and adult bone strength. These effects result from thyroid hormone

Endocrine Abstracts (2011) Vol 25
actions in bone, although a direct role for TSH in osteoblasts and osteoclasts was postulated following analysis of TSH receptor (TSHR) null mice. This hypothesis remains controversial as other studies failed to demonstrate osteoblast or osteoclast responses to TSH in vitro. Thyrostimulin is a heterodimeric glycoprotein hormone composed of α2 (GP2A) and β (GPBS) subunits that has a 10-fold higher binding-affinity for TSHR than TSH and is thought to exert paracrine effects in peripheral tissues. We hypothesized that thyrostimulin activates TSHR in bone and demonstrated expression of GP2A, GPBS and TSHR mRNAs in primary cultured osteoblasts and osteoclasts. To investigate thyrostimulin actions in vitro, we expressed the protein in Cos7 cells. Co-expressed GP2A and GPBS subunits formed heterodimers and were glycosylated and secreted appropriately. Conditioned medium from Cos7 cells expressing thyrostimulin induced a robust cAMP response in CHO cells expressing the TSHR, but no response was evident in osteoblasts or osteoclasts. To investigate thyrostimulin actions in vivo, we characterized GPBS knockout (GPBSKO) mice. In juveniles circulating T3 was normal, but T4 was reduced by 25% and TSH increased twofold. Adult GPBSKO mice were euthyroid, suggesting thyrostimulin may influence development of the hypothalamic–pituitary–thyroid axis. Consistent with the normal T3 level, endochondral ossification, linear growth and bone maturation were unaffected in GPBSKO mice. Bone micro-architecture, cortical thickness, mineral content, mineralization density and biomechanical properties were also normal in adult GPBSKO mice. In summary, although GP2A, GPBS and TSHR are expressed in bone, thyrostimulin does not induce a cAMP response in primary osteoblasts or osteoclasts. Furthermore, thyrostimulin-deficient mice display a normal skeletal phenotype indicating that thyrostimulin does not have a physiological role in bone. (GPBSKO mice were generated by Lexicon Genetics)

P9
Bone mineral density in patients with primary adrenal insufficiency compared to patients with congenital adrenal hyperplasia
Kathrin Koetz1, Manfred Ventz2, Sven Diederich1 & Marcus Quinkler1
1Charité Campus Mitte, Berlin, Germany; 2Endokrinologikum, Berlin, Germany.

Introduction
Patients with primary adrenal insufficiency (Addison’s disease) and patients with congenital adrenal hyperplasia (CAH) still tend to receive more glucocorticoids than the normal endogenous production in healthy subjects. CAH patients start glucocorticoid treatment usually with diagnosis in their early childhood, whereas Addison’s patients have a later onset of their disease and start of their treatment.

Objective
To compare patients with Addison’s disease and CAH in regard to their bone mineral density (BMD), the duration of glucocorticoid therapy and the impact of glucocorticoid pharmacogenetics.

Design, setting and participants
In a cross-sectional study patients from one university endocrine outpatient clinic were included (84 patients with Addison’s disease, 42 patients with CAH). Bone mineral density (BMD) was measured using DXA scan. Blood samples were analysed for bone resorption markers and 24 h urinary samples were analyzed for bone resorption markers.

Results
Patients with Addison’s disease were significantly older (55.1 ± 15.2 vs 39.9 ± 13.8y, P < 0.001) and taller (168 ± 10 vs 160 ± 11 cm, P < 0.001) than CAH patients, but showed no difference in BMI. Time since diagnosis was shorter in Addison’s patients (14.9 ± 10.4 vs 30.5 ± 15.5y, P < 0.001). The calculated hydrocortisone equivalent glucocorticoid dose per body surface was higher in CAH than Addison’s patients (7.2 vs 11.9 mg/m², P < 0.001). No difference was found in lumbar spine T-scores for femoral Ward’s region were lower in CAH patients (−0.96 ± 1.04 vs −0.28 ± 1.03, P < 0.005). No difference was found in lumbar spine Z-scores.

Conclusions
BMD at femoral neck and femoral Ward’s region were lower in CAH than Addison’s disease patients, indicating undesirable effects of higher glucocorticoid dose, usage of longer acting glucocorticoids, and longer duration of replacement therapy.

P10
Serum 25-OH-vitamin D levels in thalassaemia major
Ploutarchos Tzoulis, Ai Leen Ang, Farrukh Shah & Maria Barnard
The Whittington Hospital NHS Trust, London, UK.

Aims
Low vitamin D levels may contribute to the pathogenesis of bone disease in thalassaemia major patients. Our haematology department serves one of the UK’s largest populations of thalassaemia patients. We evaluated our thalassaemia major patients’ vitamin D levels and other serum bone markers.

Methods
Blood specimens were collected from all thalassaemia major patients under active follow-up in March and April 2010 and serum 25-OH-vitamin D, calcium, phosphate, alkaline phosphatase, parathyroid hormone levels were measured. The correlation of 25-OH-vitamin D with these serum markers, patient demographics and markers of iron overload was assessed. Vitamin D deficiency was defined as serum 25-OH-vitamin D < 25 nmol/l, sufficiency as 25–75 nmol/l and normal as 75–250 nmol/l.

Results
Blood specimens were collected from 74 thalassaemia major adults (38 females, 36 males) with median age of 35.5 years (range 17–58 years). Prevalence of vitamin D deficiency was 21.6%, vitamin D insufficiency 66.2% and normal vitamin D levels 12.2%. Median serum 25-OH-vitamin D levels was 39 nmol/l (range 13–113 nmol/l).

Serum calcium levels, alkaline phosphatase and parathyroid hormone were not independently associated with vitamin D levels. The only serum marker which was significantly associated with vitamin D levels was phosphate (P = 0.04), but the correlation was poor (Spearman correlation coefficient −0.243). No independent association between age or ethnic origin and vitamin D status was observed. No marker of iron overload was significantly associated to vitamin D status.

Conclusions
Most thalassaemia major patients have low serum vitamin D levels. This potentially has a significant impact on their bone health, which is already a major problem in patients with thalassaemia major. We would recommend regular measurement of vitamin D levels in all thalassaemia major patients and subsequent appropriate replacement therapy.

P11
Bone turnover markers in patients on aminobisphosphonate therapy for osteoporosis
R K Crowley1, J J Brady2, M Kilbane2 & M J McKenna1
1Department of Endocrinology, St Vincent’s Hospital, Dublin, Ireland; 2Metabolism Laboratory, St Vincent’s Hospital, Dublin, Ireland.

Aminobisphosphonates impair osteoclast cell function and reduce bone remodelling rate. It has been speculated that atypical femoral fractures in patients on bisphosphonates are the result of impaired bone remodelling. The primary aim of this prospective study was to assess levels of bone remodelling in patients with osteoporosis on aminobisphosphonate therapy by measurement of bone turnover markers. The secondary aim was to assess the adequacy of concomitant vitamin D supplementation.

We identified 77 subjects (6 males) consecutively from our endocrine practice; details of aminobisphosphonate, vitamin D and calcium therapy were recorded. The mean age of subjects was 71.3 ± 9.3 years. Mean duration of bisphosphonate therapy was 5.8 ± 4 years. Only 2 subjects suppressed bone ALP to less than the lower limit of the normal reference range, and none suppressed TRAP5b on aminobisphosphonate therapy. Mean 25OHD was 78 ± 26 nmol/l; 41 subjects had levels >75 nmol/l and only 10 had levels <50 nmol/l of whom 7 were taking 400 IU/day or less. Mean PTH was 43.9 ± 19 ng/ml, and correlated with age (r = 0.32), 25OHD level (r = −0.44) and duration of therapy (r = 0.23, P < 0.01). In conclusion, aminobisphosphonate therapy is not associated with over-suppression of bone turnover. Continuous low dose vitamin D supplementation achieved more than adequate vitamin D status if taking 800 IU daily.
**P12**

**Lithium-induced hypercalcaemia: 'past, present, and future'**

Gideon Mlawa & Sandeep Deshmukh
Southampton General Hospital, Southampton, UK.

**Background**
Lithium remains a first-line treatment for bipolar affective disorder and acute maniac states. Lithium therapy is associated with a variety of side effects including thyroid dysfunction and hypercalcaemia. Hypercalcaemia and more rarely biochemical picture resembling primary hyperparathyroidism or familial hypercalciuric hypercalcaemia may develop. Recognition of this side effect is of vital importance as an increasing number of patients with bipolar disorder are on long-term lithium therapy. We present a case report of a 65 years old lady with history of recurrent admissions with hypercalcaemia. On her last admission to the medical admission unit she was confused with increasing tiredness and slurring of speech. She had a background of bipolar disorder and was on long-term lithium therapy 300 mg bd, and depakote. Patient’s calcium level was normal prior to starting lithium treatment. The patient calcium level was 3.15 mmol, lithium level was high 2.03 mmol. ECG was unremarkable, chest X-ray and CT brain were normal. She was treated with intravenous fluids and i.v. pamidronate with improvement of her presenting symptoms. She had elevated parathyroid level (PTH) of 20 pmol/l her serum vitamin D level as well as ACE was normal. Ultrasound parathyroid and sestamibi scan were negative and 24 h urine collection was 0.54, her urine calcium creatinine ratio was <0.01. Lithium was withdrawn after consultation with psychiatrist in charge of the patient by tapering the dose to 200 mg bd, then 100 mg bd and then it was stopped. The dose of depakote was increased. Calcium level remains normal 6 months after stopping lithium.

**Conclusion**
Lithium-induced hypercalcaemia is common but underreported complication. Most patients have mild asymptomatic hypercalcaemia. This case and previously reported cases support the diagnosis of lithium induced hypercalcaemia as hypercalcaemia (hyperparathyroid state) is reversible on stopping lithium. Measurement of serum calcium and PTH levels as well as thyroid function test periodically after starting lithium treatment is advisable.

**P13**

**What predicts adverse outcomes in untreated primary hyperparathyroidism?**

Ning Yu, Peter Donnan & Graham Leese
University of Dundee, Dundee, UK.

**Context**
Rising evidence of the increased risk in mild PHPT suggests that serum calcium, which has been a main surgical criterion, maybe not an accurate indicator of disease severity or at least, not a reliable predictive factor of its long-term consequences. This study aims to identify the best biochemical predictor of adverse outcomes in untreated PHPT.

**Outcome measures and methods**
Primary outcomes considered were all-cause mortality, fatal and non-fatal cardiovascular (CVD). Secondary outcomes were nine hospital-admitted co-morbidities, including cerebrovascular disease, hypertension, renal failure, renal stones, psychiatric condition, fractures, osteoporotic fractures, cancer, and diabetes. Data was examined using survival analysis. Potential biochemical predictors tested were baseline serum calcium, PTH, creatinine and alkaline phosphatase (ALP) and other covariates considered were gender, age at diagnosis, socio-economic status, previous usage of bisphosphonates, and previous co-morbidities.

**Results**
From 1997 to 2006, we identified 2907 untreated PHPT patients (mean age, 68.4 years; 60.9% female) with a total follow-up of 7338 person-years, in Tayside, Scotland. The baseline calcium was 2.65 mmol/l and PTH was 10.3 pmol/l. In total, 648 (30.9%) patients had died during the follow up, 249 (38.4%) of fatal CVD. PTH was the only significant (indicated as P<0.05) risk factor in ALL primary and secondary outcomes observed adjusting for other covariates. Serum creatinine and ALP predicted mortality outcomes in the short term (<3 years) but not long term. In addition, high baseline serum creatinine and ALP were also associated with increased risk of hypertension, renal failure, fractures and cancer. Calcium was only associated with increased risk of all cause mortality in the short term but had no significant impact on other outcomes.

**Conclusion**
Baseline PTH, rather than calcium, predicts long-term outcomes in untreated PHPT and will have a significant impact on the justification optimal management of the condition.

**P14**

**A gene causing autosomal dominant kyphoscoliosis in an N-ethyl-N-nitrosourea (ENU) mutagenised mouse model is located on a 5 Mb interval on mouse chromosome 4 band A3**

Christopher Espai1,2, Rosie Head3,4, Holly Evans3, Gethin Thomas4, Matthew Brown4, Peter Croucher5, Roger Cox6, Steve Brown7 & Rajesh Thakker1
1University of Oxford, Oxford, Oxfordshire, UK; 2MRC Harwell, Harwell, Oxfordshire, UK; 3University of Sheffield, Sheffield, UK; 4University of Queensland, Queensland, Australia.

**Background**
Kyphosis and scoliosis are common spinal disorders that lead to significant morbidity in childhood, adolescence and adulthood. Familial and twin studies have implicated a genetic involvement, although the causative genes remain to be identified. To facilitate these studies, we investigated 12-week-old progeny of mice crossed with the chemical mutagen N-ethyl-N-nitrosourea (ENU) using phenotypic assessments that included dysmorphology, radiography and dual-energy X-ray absorptiometry (DEXA). These studies identified a mouse with fused lumbar vertebrae in association with kyphoscoliosis, designated Hvf. Inheritance testing revealed the phenotype to be transmitted as an autosomal dominant trait. Hvf mice, when compared to wild-type (WT) littermates, had: a 27% lower body weight (P<0.001); a 42% decrease in fat mass (P<0.001); a 22% reduction in lean mass (P<0.001); and a 35% increase in weight-adjusted whole body areal bone mineral density (BMD) (P<0.001). Histological analysis using haematoxylin and eosin stained sections revealed the Hvf mutant mice to have irregularly shaped vertebral bodies and displacement of intervertebral discs and ossification centres. Micro-computed tomography analysis of lumbar vertebrae from female Hvf mutant mice, when compared to WT littermates, revealed significant increases in trabecular thickness (Hvf versus WT=0.036 mm×10−2 vs 0.028 mm×10−2, P<0.01), trabecular bone volume as a proportion of tissue volume (15 vs 11%, P=0.05), and bone density (Hvf versus WT=1.27 vs 1.11 g/cm3, P<0.01). Genetic mapping localised the Hvf locus to a 5 Mb region on chromosome 4, which contains 50 genes. Thus, our studies which have established a mouse model for a monogenic form of kyphoscoliosis associated with fusion of lumbar vertebrae will help to identify a gene that has a role in the cellular and molecular mechanisms of kyphoscoliosis.

**P15**

**Parathyroid hormone concentrations in proton pump inhibitor induced hypomagnesaemia**

Amy Kennedy, Neil Gittoes & John Ayuk
Department of Endocrinology, Queen Elizabeth Hospital Birmingham, University Hospitals Birmingham, Birmingham, UK.

Severe hypomagnesaemia associated with the use of proton pump inhibitors (PPIs) is now increasingly recognised. The exact underlying mechanism is unclear but is likely to involve altered intestinal absorption of magnesium ions. Hypomagnesaemia from any cause results in functional hypoparathyroidism. PTH levels vary widely in reported cases of hypomagnesaemia associated with the use of PPIs. We examined PTH levels in patients admitted to hospital with severe (Mg<0.4 mmol/l) hypomagnesaemia and concurrent PPI use in an attempt to clarify the biochemical profile of this increasingly recognised clinical problem. Using the database of an in-house electronic prescribing system (PICS), all inpatients prescribed an oral PPI between 2005 and 2009 and with severe hypomagnesaemia were identified. Eleven patients fulfilling these criteria had concurrent PTH, serum calcium and albumin recorded. Comorbidities and other medications were also noted.

Plasma PTH range was 7.6 to 509 ng/l (median 57.2 ng/l, RR 12–65 ng/l). All 11 had hypocalcaemia (sCa 1.26–2.00 mmol/l (RR 2.1–2.6)). Two patients with the
highest plasma PTH had coexisting chronic renal failure (PTH 215.3 and 509 ng/l). In the remaining 9, PTH correlated positively with serum magnesium (r=0.85). Only one patient had the PPI stopped (although subsequently restarted within months). Six patients represented to the same hospital with a recurrent episode of hypogonadism.

PTH concentrations in PPI-induced hypogonadism vary widely and may be influenced by coexisting medical problems. The attenuated PTH response seen in most patients with PPI-induced hypogonadism does however appear to be related to the degree of hypomagnesaemia. Our results overally demonstrate that PPIs have in the past rarely been considered as a cause of hypocalcaemia/hypogonadoma and legitimise our attempt to promote awareness of this serious side effect and to clarify its biochemical pattern at presentation.

**P16**

**Radius bone loss with ageing assessed by high-resolution peripheral computed tomography differs in men and women**

Jennifer Walsh, Margaret Paggiosi & Richard Eastell
Sheffield NIHR Bone Biomedical Research Unit, Sheffield, UK.

High-resolution peripheral computed tomography (HR-pQCT) (XtremeCT, Scanco) obtains three-dimensional images of the distal radius with a resolution of 82 microns, which enables detailed study of the microarchitecture of cortical and trabecular bone. Better description of the microarchitectural changes in bone with ageing will improve understanding of which preventative and therapeutic interventions are most likely to be effective.

The aim of this study was to identify differences in cortical and trabecular bone in men and women at peak bone mass and older age.

We studied 110 male and female healthy volunteers ages 30–32 and over 70. The study was approved by the local research ethics committee. We analysed HR-pQCT images with standard software and a cortical microarchitecture programme supplied by Scanco.

Radius cortical density was lower after age 70 than at ages 30–32 in men and women (888 vs 952, 914 vs 1006 mg/cm³, P<0.001) due to higher porosity, greater pore size and lower tissue mineral density. Age differences in trabecular architecture differed between men and women. In men, trabecular thickness, but not trabecular number was lower in the older age group. In women, trabecular number, but not trabecular thickness was lower in the older age group. Older women had greater trabecular separation and trabecular inhomogeneity (the s.d. of trabecular separation) than younger women (0.25 vs 0.17 mm, P<0.01), but trabecular separation and inhomogeneity did not differ between older and younger men (0.18 vs 0.15 mm).

In conclusion, patterns of cortical bone loss with ageing are similar in men and women, but patterns of trabecular bone loss differ, with greater disruption of trabecular microarchitecture in women. It is possible that cortical bone loss is due to factors such as PTH and IGF1, which change similarly with age in men and women, and that trabecular disruption results from decreasing oestrogen levels.

**P17**

**A 5'-untranslated region mutation of the growth and differentiation factor 5 (Gdf5) gene increases expression and is associated with decreased urinary excretion of the cartilage degradation product, CTX-II: relevance to osteoarthritis**

M Andrew Nesbit1, Chris Esapa1, Rosie Head1, Katie Gaynor2, Roger Cox2, Steve Brown2 & Rajesh Thakker1
1University of Oxford, Oxford, UK; 2MRC Harwell, Oxfordshire, UK.

Osteoarthritis (OA) may be associated with endocrine disorders such as hypothryroidism, obesity, primary hyperparathyroidism or acromegaly, although often its cause remains undefined. To facilitate investigations of the underlying molecular mechanisms of OA we have investigated N-ethyl-N-nitrosourea (ENU) mutant mice using a genotype-driven approach in which candidate genes are examined for mutations. One such investigated gene is growth and differentiation factor 5 (GDF5), as in man a single nucleotide polymorphism (SNP) in its 5'-untranslated region, which reduces GDF5 expression in joints, has been reported to be associated with susceptibility to knee and hip OA. Our analysis of 10,000 DNA samples from ENU mutagenised mice identified an A/G SNP in a conserved nucleotide 47 bp downstream of the human OA susceptibility SNP and 225 bp upstream of the translation start site of the Gdf5 gene. The in vivo and in vitro effects of this polymorphism on GDF5 expression were investigated. Luciferase reporter assays of Gdf5 promoter polymorphisms in MG63 (osteoblast cells) and CH8 (cartilage cells) demonstrated that the ENU mutant (-225G) increases Gdf5 expression (1.5- and 2-fold respectively, P<0.005) in contrast to the human OA-associated polymorphism (-272T) which decreases expression (0.75- and 0.7-fold respectively, P<0.05). For in vivo studies, ENU mutant and wild-type mice were kept in accordance with national welfare guidelines and project license restrictions, aged for 14 weeks and investigated for the urinary excretion of the cartilage degradation product, CTX-II, which may be elevated in OA patients. This revealed that female homozygous mutant mice, when compared to wild-type littermates, had significantly reduced 24-h urinary CTX-II excretion (mutant 2.90±0.01 nmol/gm wild-type 4.87±0.036 nmol/gm, P<0.05) thereby suggesting that the mutant confers a protective effect against OA. Thus, we have established a mouse model with a functional alteration in Gdf5 expression that will facilitate investigation of the molecular mechanisms of OA.

**P18**

**The long-term safety and efficacy of zoledronic acid in the treatment of osteoporosis: a 3-year, randomized extension to the HORIZON-pivotal fracture trial (PFT)**

Richard Eastell1, Ian Reid2, Jane Cauley3, Steven Boonen4, Felicia Cosman5, Ping Chung Leung6, Pieter Lakatos7, Zulema Man8, Steven Cummings4, Trisha Has9, MaryEllen Ruzyczka1, Ruvie Martinez11, Guoqui Su12, Christina Bucci-Brächtwig4 & Dennis Black10
1University of Sheffield, Sheffield, UK; 2University of Auckland, Auckland, New Zealand; 3University of Pittsburgh, Pittsburgh, Pennsylvania, USA; 4Katholieke Universiteit Leuven, Leuven, Belgium; 5Helen Hayes Hospital, West Haverstraw, New York, USA; 6; Chinese University of Hong Kong, Hong Kong, China; 7Semmelweis University Medical School, Budapest, Hungary; 8Centro Tratamiento Integral Endocrinología, Buenos Aires, Argentina; 9San Francisco Coordinating Center, San Francisco, California, USA; 10University of California, San Francisco, California, USA; 11Novartis Pharmaceuticals, East Hanover, New Jersey, USA.

Treatment with a single annual infusion of zoledronic acid 5 mg (ZOL) for 3 years has been shown to be effective in increasing bone mineral density (BMD) and decreasing fractures. In order to investigate the long-term effects of ZOL, we performed a 3-year extension of the HORIZON-PFT to 6 years. A total of 1233 women who received ZOL for 3 years in the core study were randomly allocated to 3 additional years of ZOL (Z6, n=616) or blinded placebo (Z3P3, n=617). Primary endpoint was percentage change in femoral neck (FN) BMD at year 6 relative to year 3. Secondary endpoints included other BMD sites, bone turnover markers, fractures and safety. FN BMD results are shown in the figure below. In the Zb group, FN BMD remained constant in years 3-6 while it decreased slightly in the ZP3 group (between treatment difference at year 6=1.04%, 95% CI: 0.43–1.65%, P=0.0009), and similar results were seen for total hip, trochanter and lumbar spine BMD. New vertebral morphometric fractures were lower in the Zb group compared with the ZP3 group (relative risk reduction of 52%, 95% CI: 10–74%, P=0.03), while there were no differences between groups in clinical, non-vertebral, hip, and clinical vertebral fractures. Biochemical markers remained constant in the Zb group but rose slightly in the ZP3 group, remaining below pretreatment levels in both groups. The overall incidence of adverse events was similar in both groups and a numerical increase in atrial fibrillation SAEs (2.6% in Zb vs 1.1% in ZP3) was not statistically significant (P=0.26). No new safety concerns were identified. The BMD results, together with the fracture results, suggest that patients at high risk of fracture, particularly vertebral fracture, should continue on annual ZOL therapy.

**Endocrine Abstracts (2011) Vol 25**
large parathyroid adenoma on Sestamibi scan which had been managed at another hospital. On this admission, his corrected calcium was 2.79 (2.15–2.55) mmol/l, PTH 643 (15–65) pg/ml and alkaline phosphatase 258 (30–130) U/l. During his hospital stay, he developed right arm and bilateral leg weakness. MRI scan of the spine revealed a tumour causing destruction of C2 vertebral body and anterior subluxation resulting in spinal cord compression. He was transferred to the neurosurgical unit at Royal Preston Hospital where he had posterior cervical decompression and biopsy. Initial histology suggested oestis fibrosa cystica due to hyperparathyroidism, although Paget’s disease and fibrous dysplasia were considered as differential diagnoses, and the endocrinology team were therefore asked to review him. Review of a previous bone scan demonstrated increased uptake in C2 vertebral body consistent with active Paget’s and a review of the pre-operative CT and MRI imaging also confirmed Paget’s as the likely diagnosis. He was therefore treated with intravenous zoledronic acid and physiotherapy and his condition improved significantly. Subsequent histology review by a specialist bone histopathologist confirmed the diagnosis of Paget’s.

Discussion
Cervical cord compression is a relatively rare complication of Paget’s disease. In this case the correct diagnosis was delayed by the initial imaging, which suggested a bone tumour and then the histology which suggested oestis fibrosa cystica associated with co-existing hyperparathyroidism. Careful review of the previous imaging confirmed previous Pagetic involvement of the cervical spine and features on plain imaging consistent with Paget’s which allowed the correct diagnosis to be made, and appropriate treatment to be instituted.

P20
Localisation studies in primary hyperparathyroidism: our experience
Venkata Katereddy, A N B Abdul Aziz Al-akbar, Ashraf Bdiri, Andrew Ball & Khaled Ashawesh
Russells Hall Hospital, Dudley, UK.

Introduction
Primary hyperparathyroidism is not an uncommon disease with incidence of ~25–30 cases per 100 000 people, whether caused by adenoma or hyperplasia, can be cured surgically with a high rate of success. Over past decade minimally invasive surgery has become mainstay of treatment compared to traditional bilateral exploration approach. Accurate preoperative localisation of parathyroid disease is absolutely imperative for effective minimally invasive surgery.

Aim
To assess the effectiveness of ultrasound and Sestamibi scan in localising parathyroid adenoma in our hospital and compare with universal results.

Methodology and results
Retrospective study of 50 patients who underwent parathyroidectomy. Radiology results were compared with histological and operative notes. We audited 50 patients, out of which 28 were females and 22 males. Average age was 59 years and females slightly older than males.

Out of 50 patients 39 patients had primary hyperparathyroidism.
Out of 39 patients with primary hyperparathyroidism, 38/39 (97%) had ultrasound, 36/39 (92%) had Sestamibi scan.
26/38 (68%) had evidence of adenoma on ultrasound, 19/36 (53%) patients had adenoma on the Sestamibi scan. Concordance between ultrasound and Sestamibi was 33% (12/36) preoperatively.

Of 39 patients with primary hyperparathyroidism, histological findings showed, adenoma in 29/39 (75%), hyperplasia in 8/39 (20%), 1/39 (2.5%) had normal parathyroid tissue and 1 (2.5%) had no parathyroid tissue identified.

Out of 39 patients with primary hyperparathyroidism, postoperatively ultrasound localised the site of adenoma in 24/38 (63%) patients, Sestamibi localised in 20/38 (53%) patients. US and Sestamibi concordance was 29/38 (76%) postoperatively.

Conclusion
Ultrasound was slightly more accurate in localising parathyroid adenoma than Sestamibi scan in patients with primary hyperparathyroidism in our hospital. Concordance with both modalities was only 73 percent compared to 90–95% percent in various studies. Both US and Sestamibi scans should be used whenever available to localise adenomas preoperatively for patients to undergo minimally invasive surgery and prevent complications with traditional bilateral exploration.

P21
Co-existing sarcoidosis, primary hyperparathyroidism and vitamin D deficiency in a patient with hypercalcaemia
Michelle Kavanagh, Subhash Chander Rana, Nicholas Andrew Scriven, Vijay Bangar & Abdulnasim Mousa
Calderdale Royal Hospital, Halifax, West Yorkshire, UK.

Introduction
We describe a 31-year-old man with sarcoidosis found having concomitant primary hyperparathyroidism and vitamin D deficiency. Although hypercalcaemia is common in sarcoidosis it is usually accompanied by hypophosphataemia and resistance to steroids, should suggest coexisting primary hyperparathyroidism... Associated vitamin D deficiency presented management difficulties.

Case report
A 31-year-old bodybuilder presented with dyspnoea. Sarcoidosis was suspected on the basis of bi-hilar lyphadenopathy on chest X-ray. The diagnosis was confirmed by biopsy from a large axillary lymph node. Further investigations confirmed systemic respiratory and liver involvement. He had hypercalcaemia with serum calcium 3.06 mmol/l and phosphate of 0.84. He was treated with oral prednisolone with good symptomatic relief. However, his calcium remained high despite steroid treatment. His PTH ranged between 8.6 and 11.8 in the presence of high Ca confirming coexisting hyperparathyroidism. His vitamin D was <10. He was treated with vitamin D with close monitoring of Calcium, though his vitamin D is still only 43 mmol/l. He is arranged to have parathyroid adenoma localization scan in order to proceed for parathyroidectomy.

Discussion
Hypercalcaemia accompanying sarcoidosis is due to rise in circulating active vitamin D – 1,25-dihydroxycholecalciferol. Serum PTH concentration is generally low or normal. PTH remains high when there is co-existent primary hyperparathyroidism. Corticosteroid administration produces a simultaneous decline in serum calcium and 1,25-DHCC concentrations in sarcoidosis and can be used to distinguish between sarcoidosis-related hypercalcaemia and primary hyperparathyroidism. Our patient had low vitamin D which presented management difficulties as treatment with vitamin D may aggravate hypercalcaemia. The clinician, when confronts a patient with hypercalcaemia because of sarcoidosis should consider the possibility of coexistent hyperparathyroidism in the presence of high PTH and inadequate response to steroids.
Ablation of AMP-activated protein kinase (AMPK) α1 catalytic subunit in mice leads to decreased bone loss after ovariectomy and impaired bone response to intermittent PTH treatment

Mittal Shah1, Benoit Viollet1, Marta Korbontis1 & Chantal Cheun1
1Veterinary Basic Sciences, Royal Veterinary College, London, UK; 2INSERM U567, Paris, France; 3Endocrinology, Queen Mary University of London, London, UK.

AMPK is a key regulator of cellular and body energy homeostasis. We previously demonstrated that AMPK activation in osteoblasts increases bone formation in vitro while deletion of the AMPKα1 subunit leads to decreased bone mass in vivo. To determine whether bone turnover can be stimulated in the absence of AMPKα1 subunit, we subjected WT and AMPKα1−/− mice to catabolic (ovariectomy: OVX) and anabolic (intermittent PTH administration: iPTH) hormonal challenges. In vivo iPTH administration is a potent inducer of bone formation and can reverse OVX-induced bone loss. A 3-month-old female AMPKα1−/− (n=16) and WT (n=16) mice were ovariectomised. Four weeks after OVX, mice were randomly divided into two groups, one receiving saline and the other PTH (1–34) treatment (80 μg/kg per day) for 4 weeks. Tibiae were harvested from these mice and bone micro-architecture determined by micro-computed tomography. AMPKα1−/− mice displayed a decreased bone loss after OVX in the trabecular compartment. This was demonstrated by higher trabecular bone volume (+38%; P<0.01), trabecular number (+40%; P<0.001) and decreased trabecular separation (−37%; P<0.001) in AMPKα1−/− mice versus WT mice. The cortical indexes showed nonsignificant increases in bone area (+1%) and cortical thickness (+6%) in AMPKα1−/− mice versus WT mice after OVX. As expected, iPTH increased cortical and trabecular bone indexes. However, AMPKα1−/− mice showed lower trabecular bone volume (−17%; P<0.01), trabecular number (−10.4%; P<0.05), trabecular thickness (−10%; P<0.05) and increased trabecular separation (+13%; P<0.05) compared to WT mice in response to iPTH administration. The cortical indexes, bone area (−15%; P<0.01) and cortical thickness (−9.8%), were similarly lower in AMPKα1−/− mice compared to WT mice. Neither AMPKα1−/− nor WT mice bone length or body weight were altered. Overall these results demonstrate that AMPKα1−/− mice are less affected by catabolic and anabolic changes in bone turnover induced by OVX and PTH respectively, suggesting that AMPK activation plays a role in the hormonal regulation of bone remodelling.

Comparison of two high dose-bolus vitamin D regimens in women with low vitamin D levels

Ioannis Charopoulou & Steve Orme
Leeds General Infirmary, Leeds, UK.

Aim
To compare the efficacy, tolerability and safety of high doses of i.m. vitamin D2 (ergocalciferol) with oral vitamin D3 (colecalciferol) supplementation in women with low vitamin D levels.

Design and settings
Of 107 patients (25±0.50 mmol/l), aged 21–89 years were recruited in a retrospective audit. Participants were separated in two groups according to serum vitamin D levels. The Group 1 included individuals with serum vitamin D levels 30–50 mmol/l and the Group 2 with more severe depletion (<30 mmol/l). All Group 1 patients (n=65) were treated with three-doses regimen of oral monthly colecalciferol 40.000 IU (n=33) or ergocalciferol 300.000 IU bolus injection regimen (n=32). The Group 2 (n=42) received 300.000 IU oral calcitriol (n=21) or 300.000 IU i.m. ergocalciferol (n=21). The primary end points were the serum levels in 25(OH)D at 3 and 6 months for the Group 1 and at 6 weeks, 3 months and 6 months for Group 2.

Results
Oral calcitriol regimen showed significantly greater levels of 25(OH)D from i.m. ergocalciferol treatment at 6 weeks, 3 and 6 months in both groups (Group 1 P<0.008, P<0.05; Group 2 P<0.0001, P<0.01 and P<0.05). The mean difference of 25(OH)D D concentrations from baseline was significantly greater for oral calcitriol treatment at 6 weeks and 3 months in both groups. Less than 5% of patients on i.m. ergocalciferol treatment achieved levels >50 mmol/l at 6 weeks, 3 and 6 months, whereas in oral treatment, 100 and 75% of individuals obtained >50 mmol/l at 6 weeks and 3 months, respectively.

All patients in the oral calcitriol regimen with secondary hyperparathyroidism at baseline (45%, n=23) normalized their PTH levels at 3 months, whereas only 49% (41%, n=22) was corrected at 3 months, in the injection ergocalciferol regimen.

No case of hypercalcemia, vitamin D toxicity, hypercalciuria or nephrolithiasis were observed.

Conclusion
The high dose-bolus oral calcitriol treatment seems more potent than i.m. injection ergocalciferol regimen at least in the treatment of low vitamin D due to nutritional or environmental factors. The bolus 300.000 IU and the monthly 40.000 IU of oral calcitriol produced higher and sustained increase in serum 25(OH)D.

Management of primary hyperparathyroidism

Anna Irina Palalaiu & Diana Raskauskiene
Walsall Manor Hospital, Walsall, West Midlands, UK.

Primary hyperparathyroidism (PHPT) is a common reason for referral to the endocrinology team. We aimed to estimate the prevalence of associated vitamin D deficiency and osteoporosis, to evaluate the usefulness of imaging studies in identifying a parathyroid adenoma prior to surgery and to assess response to surgical treatment in the setting of a District General Hospital.

We searched our outpatient clinic letters and identified 64 patients diagnosed with PHPT between July 1996 and May 2009. Thirty-four (53.1%) patients were of white British ethnic origin and 3 (4.7%) were of Asian Pakistani origin. Mean age (±s.d.) was 61.0 (+±13.7) with 50 patients under the age of 50. The mean (±s.d.) highest recorded calcium was 2.95 (±0.19) mmol/l. Eleven patients had a calcium level above 3 at diagnosis and during follow-up the level rose above 3 in 20 patients. Vitamin D levels were measured in 44 patients; 11 (25%) had levels between 10 and 15 μg/l and 9 (20%) levels below 10 μg/l. Bone densitometry scans were done in 22 patients; 7 (31.8%) had osteopaenia and 12 (54.5%) had osteoporosis. The age range of patients with osteoporosis was 38–86 (mean 63). Twenty-three patients received surgical treatment (4 in our hospital, 17 in a tertiary referral centre and 2 elsewhere). Following surgery 21 (91.3%) patients had normal calcium levels. In 5 patients in whom we could correlate the report of a Tc sestamibi scan with surgical findings an adenoma was correctly identified in 2 (40%) patients by the isotope scan. Neck ultrasound correctly identified an adenoma in 3 (60%) of patients.

In our cohort of patients with primary hyperparathyroidism there was a significant incidence of vitamin D deficiency and osteoporosis. Based on our data, D risk screening for these conditions is advisable in patients being assessed for PHPT.
Results

<table>
<thead>
<tr>
<th>Vitamin D level</th>
<th>Urinary calcium creatinine at diagnosis</th>
<th>Urinary calcium creatinine at any time</th>
<th>DECA scan</th>
<th>Retinol/ vitamin A stored scan/ Serum vitamin A prior to surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested</td>
<td>n=45/54 (87% 82%)</td>
<td>n=50 (86%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusion
The diagnostic work-up for PHPT needs to be improved to achieve full compliance with national guidelines. This could be achieved with the use of an out-patient proforma and requesting a panel of bloods (e.g. 'PHPT Panel') rather than individual tests. Vitamin D supplementation is beneficial for the treatment of PHPT.

Clinical biochemistry

P27
A semi-automated method for measuring salivary cortisol and cortisone by tandem mass spectrometry with sample extraction
Laura Owen, Rachel Jones & Brian Keevil
University Hospital of South Manchester, Manchester, UK.

Introduction
There has been much interest recently in measuring both salivary cortisol and cortisone due to the presence of 11b-hydroxysteroid dehydrogenase type 2 enzyme in the salivary glands. This enzyme facilitates the conversion of cortisol to cortisone; hence the concentration of cortisone in saliva may also be of interest. Studies have shown recently that salivary cortisol and cortisone are good markers of serum free cortisol status.

Methods
Calibrator, quality control or sample (50 µl), 10 µl internal standard (D4 cortisol) and 150 µl water were added to wells of 96 deep-well plate. Online solid phase extraction (SPE) of 150 µl of this was performed using the Spark Holland Symbiosis in XLC mode (eXtraction Liquid Chromatography) with HySphere C18 HD 7 µm cartridges. Chromatography was performed using a Phenomenex Onyx Monolithic C18 column (25 X 4.6 mm).

Results
Retention times using the chromatography conditions was 2.22 min for cortisol and 1.98 min for cortisone with resolution of the two compounds. The limits of detection were 0.75 nmol/l for cortisol and 0.5 nmol/l minutes for cortisone. No ion suppression was observed 10 patient samples tested using Sarstedt Salivettes for cortisol (blue top), however green top Salivettes were found to be unsuitable due to a large background signal. Extraction cartridges were reliable for up to 15 uses.

Discussion
We have developed a novel assay for measuring salivary cortisol and cortisone in saliva utilising semi-automated solid phase extraction. This allows a sample clean-up step, which improves upon a previously published assay without adding to the length of time required to prepare an assay. We have found that the blue top Salivettes for cortisol are the preferred device for collecting samples for this assay.

P28
25OH vitamin D analysis by liquid chromatography tandem mass spectrometry: interpret results with caution
Michael Wright1, Kevin Taylor1, Deborah Mawson2, Philip Grace2 & David Halsall3
1Department of Clinical Biochemistry, Addenbrooke’s Hospital, Cambridge, UK; 2HFL Sport Science Ltd, Cambridgeshire, UK.

Liquid chromatography tandem mass spectrometry (LC-MS/MS) methods are considered superior to immunoassay for the analysis of serum 25-hydroxy vitamin D (25-(OH)D) due to improved performance and potential cost benefits. As LC-MS/MS is appropriate for the analysis of other clinically relevant hormones tandem mass spectrometers are becoming commonplace in UK clinical laboratories. However, like immunoassay, LC-MS/MS methods are not foolproof and inappropriate use of LC-MS/MS methods can have an adverse impact on patient care. Triple quadrupole mass spectrometers allow the monitoring of several fragment ions derived from a single molecular ion. This allows quantification using one fragment (the quantifier) and analysis of other fragments (qualifiers) to confirm the identity of the molecular ion. Examples are provided as to the use of qualifier ions to detect both endogenous and exogenous interferences that would otherwise bias LC-MS/MS 25-OHD methods. During optimisation of an LC-MS/MS/MS-based 25-OHD method, the use of qualifier ions detected an endemical interference which was significant in 1023 of the 3060 patient samples studied using a commonly used quantifier ion of m/z 211. We identified three cases that were classified as severely 25-OHD deficient (<12.5 nmol/l) using a 383-237 transition which would be classified as vitamin D replete (>50 nmol/l) using the 383-211 transition. The interference was still present despite use of a variety of sample preparation methods (liquid/liquid extraction, off-line and on-line solid phase extraction). Accurate mass MS studies show the interference to have a mono-isotopic m/z of 383.2964 compared to m/z 383.3308 for 25-OHD. Further studies suggest that this is an exogenous compound, probably exuded from the sample collection tubes. The isobaric C-3 epimer of 25-OHD [3-epi-25(OH)D3] has also been shown to be a potential interference in LC-MS/MS based serum 25-OHD assays. The use of appropriate qualifier ions to rule out the presence of 3-epi-25(OH)D3 is demonstrated.

Clinical biochemistry

P29
Turbulent flow liquid chromatography—tandem mass spectrometry for the analysis of bio-available testosterone in serum
Michael Wright1, Lewis Couchman2 & David Halsall3
1Department of Clinical Biochemistry, Addenbrooke’s Hospital, Cambridge, UK; 2King’s College Hospital, London, UK.

Testosterone in serum may be unbound (free), or bound to either sex hormone binding globulin (SHBG) or albumin. Consequently, total serum testosterone analysis may be misleading in situations where binding protein concentrations are abnormal. Current methods for estimating the biologically active (bio-available) serum testosterone concentration involve physical separation of testosterone fractions and are not amenable to high-throughput analysis. The use of automated immunoassays for total serum testosterone has also been questioned. Liquid chromatography—tandem mass spectrometric (LC-MS/MS) methods for measuring testosterone have gained favour due to their superior selectivity. The separation of testosterone fractions by turbulent flow chromatography (TFC – Turbulent Flow technology, ThermoFisher Scientific) prior to MS/MS analysis was investigated. TFC is based on the direct injection of biological samples onto a column packed with relatively large particles at high flow-rates. In the resulting turbulent flow, smaller analytes can enter the column interstices but larger proteinaceous material is excluded. Serum from a male volunteer was analysed (i) following protein precipitation with equal volumes of methanol (total testosterone) and (ii) by direct injection onto the TFC column (retained fraction). By comparison of peak areas, it was found that 25% of the total serum testosterone was retained after direct injection. This agrees well with an ammonium sulfate precipitation method for bio-available testosterone (22%) used on the same sample. Based on the known binding affinities of SHBG and albumin for testosterone, it is likely that the more weakly bound albumin fraction was, to some degree, dissociated during the TFC process. Testosterone and SHBG were added separately to serum pools and analysed as previously described. The peak area ratio was independent of total testosterone concentration, but decreased at higher SHBG concentrations. Whilst further validation is required to prove the utility of TFC, it has considerable potential for the analysis of bio-available steroid hormones in serum.
In 2006, he was referred to another hospital with nocturnal sweating and tremors. He was found to have a left sided phaeochromocytoma which was removed laparoscopically with uneventful follow up. Unfortunately no initial results or histology were available on referral to our department.

Follow up in primary care had involved annual 24 h urine catecholamines but no imaging. The patient had remained well with no recurrence of his symptoms and stable blood pressure. Routine annual collection in 2010 revealed a striking abnormality in biochemistry. On examination the patient was well and reported no symptomatology with blood pressure 125/73 mmHg.

He was urgently referred for management of his recurrent phaeochromocytoma. Plasma metanephrines were reassuringly normal with normetadrenaline 0.48 nmol/l and metadrenaline 0.16 nmol/l (<1.3 and <0.7 nmol/l respectively indicate low probability of phaeochromocytoma).

On direct questioning of the patient he had been diagnosed earlier in the year with Parkinson’s disease and started on co-Beneldopa with the dose gradually titrated up.

Drug interactions in the case of investigation of phaeochromocytoma with 24 h urine catecholamines are well documented. In this case the cause for a sudden and dramatic rise in the urinary catecholamines was revealed by detailed history taking and spared the gentleman unnecessary further investigation. Questioning of changes in medication and dosing should be routine in every clinical assessment. We will be following up this gentleman with annual plasma metanephrines.

P31
Seasonal variation of vitamin D level among menopausal and postmenopausal Saudi women
Waleed Tamimi, Raad Kanaan, Myson Adham & Salih Alijasser
College of Applied Medical Sciences and College of Medicine, King Saud Bin Abdulaziz for Health Science, King Abdulaziz Medical City, Riyadh, Saudi Arabia.

Objective
Vitamin D deficiency is common among women despite abundant sunshine in Saudi Arabia. No local study has evaluated the seasonal vitamin D level among hospitalized menopausal and postmenopausal Saudi women. The aim of this study was to estimate the prevalence of vitamin D deficiency among menopausal and postmenopausal in Saudi Arabia during summer and winter.

Methods
Data were retrospectively collected for 1556 female patients (>19 years) during their hospital visits between January 2009 and December 2009 in Riyadh, Saudi Arabia. The patients were divided into two groups 659 (42%) and 897 (58%) patients during summer and winter respectively. The summer group was subdivided into menopausal 425 (27%) (19–49 years) and postmenopausal 234 (15%) (≥50 years); and in winter, 543 (35%) and 354 (23%) respectively. Serum levels of 25-hydroxy vitamin D (25OH VIT D) were measured by HPLC method.

Results
The prevalence of hypovitaminosis (<0.50 nmol/l) in menopausal and postmenopausal women was 18% and 7.6% during summer; 26% and 14% during winter respectively. There was a significant difference between the mean of vitamin D level of menopausal (33.3 ± 3.3) and postmenopausal (44.4 ± 35) women during summer (P < 0.0001) and (28.5 ± 27) and (36.3 ± 28) during winter (P < 0.0001) respectively. The between and within seasonal vitamin D variations were observed among menopausal and postmenopausal women (P < 0.05). The between seasonal variations represented a 14% increase in vitamin D level for menopausal and 18.5% for postmenopausal women and that for within seasonal variation were 25 and 21% increase respectively.

Conclusion
A higher prevalence of vitamin D deficiency among the hospitalized menopausal and postmenopausal Saudi women was observed in winter. Seasonal variation was also observed between and within this population. Clinicians should consider determination of vitamin D level in these women especially menopausal and advice proper supplementations.

ICR was <1 (Mean-0.05) in all cases except in two cases (exogenous insulin administration and insulin autoimmune syndrome (IAS)). In the former, insulin was elevated (445 pmol/l) with undetectable C-peptide levels (< 94 pmol/l). In the patient with IAS, insulin levels was disproportionately high (17 750 pmol/l) along with corresponding high levels of C-peptide levels (8520 pmol/l) i.e. ICR > 1.

Conclusion
When exogenous insulin administration is excluded, the reversal of ICR (normally <1) can be considered as a surrogate marker for causes associated with elevated insulin antibody levels. This may negate the necessity of measuring insulin antibody levels in all cases of endogenous hyperinsulinemia.

P32
Significance of insulin-C-peptide ratio in the diagnostic algorithm for endogenous hyperinsulinemia
Suma Sugunendran1, Deepa Narayanan1, Waqas Shafiq2, Mohamed Malik2 & Thozhukat Sathyapalan3
1 Hull and East Yorkshire Hospitals NHS Trust, Hull, UK; 2 Scunthorpe District General Hospital, Scunthorpe, UK; 3 Hull York Medical School, Hull, UK.

Background
Insulin, derived from a single chain precursor pro-insulin, is cleaved by convertases to form insulin (1/2 – 4 min) and C-peptide (1/2 – 30 min). The ratio of insulin to C-peptide levels, which is usually less than one, is reversed in presence of exogenous insulin and insulin autoimmune syndrome. We present the significance of insulin-C-peptide ratio (ICR) in the diagnostic algorithm for the investigation of endogenous hyperinsulinemia.

Methods
All requests for measurement of insulin and C-peptide levels were retrospectively reviewed over a 4 years period from 2006 to 2009 from two hospitals. Information regarding the indications for the requisitions, the clinical history and the final diagnosis was retrieved after review of the clinical notes.

Results
Of 52 samples from 21 patients were analysed. 11/21 patients were being investigated for hypoglycaemia (Table 1) and 10 patients were investigated for determining the type of diabetes mellitus.

Table 1 Causes of hypoglycaemia.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulinoma</td>
<td>1</td>
</tr>
<tr>
<td>Endogenous insulin*</td>
<td>1</td>
</tr>
<tr>
<td>Exogenous insulin use</td>
<td>1</td>
</tr>
<tr>
<td>Insulin autoimmune syndrome (IAS)</td>
<td>1</td>
</tr>
<tr>
<td>Miscellaneous causes†</td>
<td>2</td>
</tr>
<tr>
<td>Hypoglycaemia not achieved</td>
<td>5</td>
</tr>
</tbody>
</table>

*Incomplete investigation- patient died due to gastric adenocarcinoma.
†Ca esophagus + poor nutrition, renal failure + diabetes.

P33
Cross reactivity of Spironolactone with androstenedione immunoassay
Venkata Katreddy, Ashraf Bdiri & Khaled Ashawesh
Russells Hall Hospital, Dudley, West Midlands, UK.

Introduction
Hirsutism, the presence of terminal (coarse) hairs that appear in a male-like pattern, affects 5–10% of women and may cause concern for an underlying endocrine disorder or malignancy. Spironolactone compete for the androgen receptor in the hair follicle, therefore, it is frequently used in treatment of hirsutism. We report a case, in which treatment of hirsutism with Spironolactone
interfered with androstenedione immunoassay and raised unnecessary concern about androgen secreting tumour.

Case

A 24-year-old lady was referred to our endocrine clinic with history of excessive hair growth since age of 16, with significant psychological impact. She had no significant past medical history, apart from coil insertion for contraception. Her initial investigations revealed normal testosterone, FSH, LH, prolactin, thyroid function. US pelvis revealed polycystic ovaries. Diagnosis of polycystic ovary syndrome was made and was subsequently treated with Spironolactone BD, which further increased to 100 mg BD in 6 weeks time, and referred for funding for laser treatment. A repeat hormonal profile, 3 months later, showed elevated androstenedione at 59.5 nmol/l (1–11.5 nmol/l), with normal DHEAS, testosterone, LH, FSH, estradiol and SHBG. On repeat testing in a month, androstenedione was high at 80.5 nmol/l, while other androgens remained within normal range. Because of a very high androstenedione level, MRI adrenals and ovaries were arranged to rule out androgen producing tumour; this was unremarkable except for polycystic ovaries. At this stage, Spironolactone interference in the immunoassay of androstenedione was suspected and Spironolactone was subsequently stopped. Few months later, on a repeat testing, androstenedione level returned to normal.

Discussion

Cross reactivity of Spironolactone or its metabolites with the immunoassay of androstenedione has previously been reported. It is important, therefore, for the clinicians treating patients with Spironolactone for Hirsutism to be aware of this and communicate with biochemist to prevent any unwarranted investigations and distress for patients.

P34

An unusual case of leg weakness in a patient with diabetes and Addison’s disease (Is there a common link?)

Julia Platt, Maitrayee Choudhury, Alexis Manning & Nicholas Withenshaw

University Hospital Llandough, Cardiff, UK.

Both diabetes and Addisons’ are conditions which can be associated with muscle weakness and altered potassium levels. This is a case of recurrent muscle paralysis in a patient with both conditions with the underlying abnormality being the neurological condition of hypokalemic periodic paralysis. A 23-year-old Caucasian gentleman was admitted to medical admissions with sudden onset weakness of his lower legs. On examination he had reduced power of his hips and knees, with intact sensation. The main finding from his blood tests was a serum potassium level of 3.2 mmol/l. He had a past medical history of poorly controlled type 1 diabetes and Addisons’ disease. His weakness improved following a short course of oral potassium replacement. He was discharged home but readmitted 2 days later with similar symptoms and potassium of 2.9 with. Autonumone markers, thyroid function tests and plasma renin levels were normal. He underwent an EMG study, which revealed abnormalities showing reduced myotonic discharges, which supported a diagnosis of hypokalemic periodic paralysis.

This is an autosomal dominant condition characterised by intermittent episodes of flaccid paralysis with associated hypokalaemia, which are reversible. The genetic defect leads to an alteration in voltage gated sodium and calcium channels in the skeletal muscle. Rare associations have been made with diabetes and hypokalaemic periodic paralysis, which has been triggered by insulin administration. Hypokalaemia may also be precipitated by excessive corticosteroid or excessive mineralocorticoid replacement. This case raises some interesting and challenging questions regarding the complex management of the condition in view of the electrolyte abnormalities in the background of his medical conditions and whether there may a genetic link underlying all three diseases.

P35

The short Synacthen test (SST): should we be testing at both 30 and 60 min?

Richard Carroll, Jalini Joharattano & Jeannie Todd

Imperial College Healthcare NHS Trust, London, UK.

The short Synacthen test (SST) is used to evaluate adrenal glucocorticoid secretion in response to synthetic ACTH (also known as tetraocortis or Synacthen). Traditionally, after a baseline cortisol and ACTH, two plasma cortisol samples have been taken after i.v. or i.m. administration of Synacthen 250 μg, one at 30 min and one at 60 min. However practice varies and some physicians only take one cortisol measurement at 30 min.

Hypothesis

A protocol involving one assessment of plasma cortisol levels post Synacthen administration is as effective at confirming hypoadrenalism as the standard two sample protocol.

We studied 101 patients who had undergone a SST at Hammersmith Hospital between January 2009 and Nov 2010. We accepted a post Synacthen cortisol level of ≥ 580 nmol/l (≥ 440 μg/dl) on new Abbott immunoassay as cortisol levels on this assay are reported to be 20% lower as evidence of an intact HPA axis. Overall 88% showed an adequate response (ie passed) on SST. Of this group 100% passed the SST based on the 60 min cortisol value alone. 95% passed if the 30 min cortisol value alone was assessed, giving a false negative (i.e. failed test) rate of 5%. Reduction of the upper range of normal for the 30 min time point to ≥ 444 nmol/l (≥ 355 nmol/l on Abbott immunoassay) provided a sensitivity of 100% with respect to the full protocol results, and did not provide any false negatives or false positives.

We conclude that a modified SST protocol could be based on either a sole 60 min post Synacthen cortisol level assessment, or a sole 30 min post Synacthen cortisol assessment with an adjusted lower cut off at this time point. A retrospective audit of 100 further SSTs is planned to check the performance of both these strategies.

P36

Comparison of serum cortisol measurement by immunoassay and liquid chromatography–tandem mass spectrometry in patients receiving the 11β-hydroxylase inhibitor metyrapone

Phillip Monaghan1, Laura Owen2, Peter Trainer1, Georg Brabant1, Bridie Keenill & Denise Darby1

1The Christie NHS Foundation Trust, Manchester, UK; 2University Hospital of South Manchester, Manchester, UK.

The accurate measurement of cortisol by immunoassay is compromised by the potential for cross-reactivity of reagent antibodies with structurally-related steroid compounds present in patient serum. This susceptibility is potentiated when normal steroid metabolism is altered pharmacologically by anti-steroidogenic drugs. This class of drug is utilised in the management of Cushing’s syndrome to moderate cortisol production. To investigate the effect of the 11β-hydroxylase inhibitor metyrapone on serum cortisol assay, a comparison of measurement by immunoassay versus liquid chromatography–tandem mass spectrometry (LC–MS/MS) as gold standard was conducted. Cortisol was measured in serum from three patient groups; i) patients receiving metyrapone therapy (n=112), ii) control group of patients diagnosed with Cushing’s syndrome currently receiving no treatment (n=32) and iii) control group of normal patients with no known adrenal pathology and not receiving medication known to interfere in the immunoassay of cortisol (n=79).

Bland-Altman plots showed good agreement between methods for the normal control group (bias = −2.2% (4.4 nmol/l)) and Cushing’s control group (bias = +1.3% (3.5 nmol/l)). This was in contrast to the metyrapone therapy group (bias = −23% (−59 nmol/l)). Passing-Bablok linear regression revealed bias observations were constant in nature with minimal contribution from proportional error. Pearson correlation coefficients for the three patients groups were r = 0.39*, 0.17 and 0.36* for the normal control, Cushing’s control and metyrapone therapy groups, respectively (*indicates Pearson correlation coefficient significant at P<0.05). LC–MS/MS results were positively correlated with the difference. Further interrogation of metyrapone group data showed the degree of difference between LC–MS/MS versus immunoassay positively correlated with dose. This trend was also noted for serum 11-deoxycorticisol concentration with dose.

In conclusion, these data show that the liability of immunoassay measurement of serum cortisol to interference in patients receiving metyrapone may lead to erroneous clinical decisions concerning dose titration.

P37

Polycythaemia in men treated with transdermal and intramuscular testosterone

Tomas Agustsson, Barbara McGowan, Jake Powrie, Stephen Thomas & Paul Carroll

Department of Diabetes and Endocrinology, Guy’s and St Thomas’ Foundation NHS Trust, London, UK.

Background

Testosterone replacement therapy has been shown to produce a wide range of benefits for men with hypogonadism with studies showing improvement in libido,
bone density, muscle mass, body composition, mood, cognition, and erythropoiesis. The risks associated with testosterone replacement therapy are less well characterised and there is a lack of larger randomised trials. One recognised risk is polycythaemia. The aim of this study is to assess the frequency of polycythaemia in men treated with testosterone and to compare it’s frequency with different treatment modalities.

Methods
This is a retrospective observational study. We analysed biochemical and haematological parameters of all men on testosterone therapy who attended our endocrinology unit from the 1st of January 2009 until the 30th of June 2010. Of a total of 173 men, 86 (50%) were treated with testosterone undecanoate (Nebido), 57 (33%) with transdermal testosterone gel, and 30 with intramuscular testosterone in the form of Sustanon. Data were collected on haemoglobin concentrations and packed cell volumes. Polycythaemia was defined as haemoglobin concentration >17 g/dl or packed cell volume >5.05.

Results
Out of the 173 men 25 (14.5%) developed polycythaemia on at least one blood sample during the above period. 12 of the 86 men treated with Nebido (14%), 8 out of the 57 men treated with transdermal gel (14%), and 5 of the 30 men treated with Sustanon (17%) developed polycythaemia. There was therefore no significant difference between the different treatment groups. This demonstrates the importance of careful monitoring of haematological variables during any testosterone treatment so that appropriate measures can be taken if erythrocytosis occurs.

Conclusion
In our experience polycythaemia is a common risk with any testosterone replacement therapy. Although previous studies have indicated that this is less likely with transdermal testosterone, risk factors need to be equalised. This study shows the risk to be equal across treatment groups. This demonstrates the importance of careful monitoring of haematological variables during any testosterone treatment so that appropriate measures can be taken if erythrocytosis occurs.

P38
Hypocalcaemia presenting via an acute medical admissions unit is only rarely adequately investigated
Rebecca Griffiths1, Stewart Pattman2 & Richard Quinton1
1Endocrine Research Group, Institute of Human Genetics, Newcastle upon Tyne, UK; 2Newcastle upon Tyne NHS Hospitals Trust, Newcastle upon Tyne, UK.

Aim
To determine whether cases of hypocalcaemia presenting via an emergency medical admissions unit (EAU) are appropriately investigated.

Background
Hypocalcaemia is a potentially life threatening abnormality, with a prevalence of 1% among hospital inpatients.1,2 Risk factors include vitamin D deficiency, renal disease, hypoparathyroidism (typically post-neck surgery) and hypomagnesaemia. A reasonable investigational dataset comprises 25OHVitD, PTH, Mg, phosphate (P) and alkaline phosphatase (ALP).

Methods
The biochemistry database was interrogated for the year beginning 1st December 2008 to identify EAU patients with aCa checked on EAU, with only a few cases reported. However, in view of the great risks posed to both mother and baby we recommend regular monitoring of serum sodium levels in all patients with pre-eclampsia.

Conclusions
Hypocalcaemia is less prevalent among patients presenting via EAU than in hospital inpatients, so the resource implications of investigating it properly are not huge. Yet this is not happening reliably, despite the high percentage of abnormal results when tests are done. Owing to delayed/incomplete investigation, therapeutic interventions will necessarily be delayed/omitted and hospital admission likely to be correspondingly prolonged. Guidelines are in development to improve understanding of this issue.
where newer treatment options are becoming available and greater importance is attached to length of hospital stay.

P41
Tanned and pregnant—primary adrenal insufficiency during pregnancy
Naik Haya & Ramzi Ajjan
Leeds Teaching Hospitals, Leeds, UK.

Primary adrenal failure is a rare condition with an incidence during pregnancy of around 1 case in 12 000 gestations. A 31 years old primi gravida presented 10 days after giving birth to a healthy child at full term with postural dizziness and severe fatigue. There was no history of excessive blood loss during delivery and her past medical history was unremarkable, but low blood pressure (BP) was reported throughout pregnancy. She mentioned increased body pigmentation over the past year and had a severely pigmented scar on her leg with normal buccal mucosa. BP was 80/50 mmHg with a significant postural drop. She had low sodium (127 mmol/l) and raised potassium (5.7 mmol/l), associated with dehydration (urea 11.6 mmol/l) and marginally low bicarbonate (21 mmol/l). A synacthen test failed to show a cortisol response (0 and 30 min cortisol < 50 mmol/l) and adrenal antibodies were positive with elevated ACTH at 1075 ng/l consistent with primary adrenal failure. Her endocrine investigations showed low IGF1, normal prolactin (548 IU/l) with undetectable oestradiol, FSH and LH. FT4 was 9.4 pmol/l and TSH 1.0 mU/l. Given her normal prolactin with low IGF1 and FT4, the possibility of associated hypophysitis was considered, although abnormal TFTs may have been indicating sick euthyroid syndrome. She was immediately treated with hydrocortisone and fludrocortisone was added 2 weeks later resulting in complete resolution of her symptoms. Endocrine testing 2 weeks after initial presentation showed FSH 3.4 IU/l, LH 2.7 IU/l, oestradiol 146 pmol/l, FT4 15 pmol/l, TSH 1.2 mU/l IGF1 30.2 nmol/l with normal U&Es. 2 weeks later resulting in complete resolution of her symptoms. Endocrine testing 2 weeks after initial presentation showed FSH 3.4 IU/l, LH 2.7 IU/l, oestradiol 146 pmol/l, FT4 15 pmol/l, TSH 1.2 mU/l IGF1 30.2 nmol/l with normal U&Es. In summary, we present a case of primary adrenal failure with classical symptoms during pregnancy but with the diagnosis made in the postpartum period. The patient had undergone a largely uneventful pregnancy and delivered a healthy child at full term. Her low IGF1 and deranged TFTs at presentation normalised following hydrocortisone replacement therapy.

P42
A review of the Endocrine Transition Service over the last 10 years
Nilanjana Ray1, Tanja Davison1, Fiona Ryan1, John Wass2 & Niki Karavitaki2
1Department of Paediatric Endocrinology, John Radcliffe Hospital, Oxford, UK; 2Department of Endocrinology, Churchill Hospital, Oxford, UK.

Poor transitional care leads to increased loss to follow up, non-adherence to treatment, high morbidity and mortality. The paediatric and adult endocrinology teams in our Trust have been running a joint transition service since 10/2000. A review of this service was undertaken, in order to examine its effectiveness and to aid its improvement. The details of all 81 patients, who had been through transitional care between 10/00-09/09 were acquired. Their records were reviewed for relevant information. A questionnaire and covering letter were sent to survey patient satisfaction and their views on their transition process. The mean age of transition was 18.6 years. Currently, 34 out of 77 (44%) patients have replied to the questionnaire (4 had died). 79% felt that the timing of transition was appropriate, 100% that it was helpful to meet the adult endocrinologist prior to transfer and 12% that they would prefer two transition clinic appointments. 76% reported that they were given enough information about their condition and the transition process and 53% that they felt confident to be seen without their parents present in 1st or 2nd appointment in the adult clinic. The mortality rate was 5%, 4% of the patients had been admitted acutely within 2 years of transition and 11% had issues with adherence to treatment. The DNA rate at the second adult follow-up appointment was 16% (for general adult endocrine clinics 6–10%). Overall, the patient satisfaction with the service is high. Providing written information about the transition process and the conditions affecting the patients and addressing compliance issues will further improve it. Generation of national guidelines on endocrine transition care is of major importance for the continuing wellbeing of the patients and their willingness to take responsibility of their own health and to comply with health support and treatment.

P43
Tolvaptan in a patient with hyponatraemia and a normal chest X-ray
Hema Venkataraman & Zayd Merza
Barnsley Hospital NHS Foundation Trust, Barnsley, UK.

A 61-year-old man was referred to the Endocrinology clinic with a 5-month history of hyponatraemia. It was first detected as part of a routine test, which revealed a serum sodium of 124 mmol/l. His serum osmolality was 253 mOsm/kg, urine sodium was 32 mmol/l and urine osmolality 317 mOsm/kg. His results suggested SIADH. He already had a chest X-ray and brain MRI scan done which were reported as normal. His thyroid function test and short synacthen test were normal. He had been commenced on fluid restriction and demeclocycline, however his sodium remained low with a mean of 122 ± 3.4 mmol/l and when seen in clinic it was 118 mmol/l. Accordingly a CT scan of his chest was arranged. The patient was admitted to hospital and commenced on Tolvaptan (a selective vasopressin V2-receptor antagonist). After only two doses of 15 mg/day his sodium came up to 132 mmol/l within 48 h and the Tolvaptan was stopped. Over the next 6 days his sodium remained stable between 128 and 133 mmol/l and he was discharged on fluid restriction. His CT scan revealed a 2.2 cm lesion in his left lung and a biopsy confirmed small cell carcinoma. Nine days after discharge a repeat test showed his sodium was down again to 116 mmol/l.

This case illustrates the importance of excluding an underlying malignancy in a patient with significant hyponatraemia even if the chest X-ray is reported as normal. It also demonstrates the efficacy of Tolvaptan in rectifying hyponatraemia in SIADH.

P44
A case of thyrotropinoma co-secretion FSH and LH
U Y Raja, M A Karamat, Sadaf U Nasah, Lisa Shephard & Asad Rahim
Birmingham Heartlands Hospital, Birmingham, West Midlands, UK.

Introduction
Thyrotropinomas are rare pituitary tumour accounting for 0.5-1.0% of all pituitary tumours. There are only seven reported cases of mixed TSH/LH/FSH producing adenoma. We report a patient with secondary hyperthyroidism due to TSH producing adenoma co-secreting FSH and LH.

Case report
A 75-year-old Cascanic male was seen with 2-year history of tiredness, anxiety and palpitations along with 7-month history of weight loss. He also complained of impotence for 2 years along with lack of sex drive. His thyroid function tests showed FT4 level of 55.1 pmol/l along with TSH level of 8.3 mU/l and anti TPO antibodies of 7 IU/ml. His other anterior pituitary tests were: FSH 3.7 IU/l, LH 3.0 IU/l, IGF1 < 3.2 nmol/l and prolactin level of 389 mU/l. His alpha Sub Unit came back high at 1.95 IU/l and pituitary MRI showed a 1.8 by 2.5 cm lesion in the pituitary gland compressing the right optic nerve and invading the right cavernous sinus. He was started on octreotide LAR 60 mg along with carbimazole 30 mg once daily and following discussion in pituitary MDT meeting was referred for trans-sphenoidal pituitary surgery. Following pituitary surgery his TFT came back as FT4 of 26.2 pmol/l with TSH of 3.5 mU/l. Biopsy of the lesion confirmed mixed TSH/LH/FSH producing adenoma.

Discussion
Thyrotropinomas are rare with a prevalence of about one case/million. Most cases are found in the fifth-sixth decade of life. Around 30% of TSH producing adenoma also co-secrete other pituitary hormones most commonly GH and prolactin. Majority of thyrotropinomas are macroadenomas (90%) and clinical features of hyperthyroidism are usually present. Definite treatment consists of pituitary surgery though octreotide therapy lead to normalization of thyroid hormones in about 70% of cases with partial shrinkage of tumour in 40% of thyrotropinomas.
Introduction

Adrenal incidentaloma is defined as a clinically innocent adrenal mass that is incidentally detected after imaging studies conducted for reasons other than the evaluation of the adrenal glands. Existing guidance suggests that excess catecholamine and cortisol secretion should be ruled out in all cases and excess aldosterone secretion should be ruled out hypertensive patients. Certain features on CT scanning such as a Hounsfield value of over 10 are useful in differentiating between incidentaloma and other lesions. In addition repeat evaluation after a period of time is suggested.

We audited how adrenal incidentaloma are investigated in a large district general hospital in the North West of England.

Method

We reviewed abdominal CT scan reports from 2694 patients who had their scans between August 2007 and February 2010. Patients with adrenal incidentaloma were identified and their clinical records were reviewed.

Results

Adrenal incidentaloma were reported in 93 patients giving rise to an approximate prevalence of 3.45%. Thirty-four of the scans were requested by physicians. Forty-five percent of the lesions were left sided and 26% had bilateral lesions. The size of the lesion was reported in 68 scans with the largest being 6 cm. The average size was 1.68 cm. The Hounsfield value was reported in 13 scans and it was more than 10 in 6 scans. Only five patients had excess catecholamine secretion ruled out and only three had excess cortisol secretion ruled out. Only two patients had excess aldosterone secretion ruled out.

Only twelve patients had repeat imaging done and only three had their biochemistry repeated.

Discussion

Adrenal incidentaloma are inadequately managed by non specialists. The quality of radiology reports with regard to Adrenal incidentaloma is inadequate. This deficiency may be improved by increasing the awareness of the importance of investigating these lesions amongst non-endocrinologists.

Case report

A 67-year-old female with untreated congenital adrenal hyperplasia (21-hydroxylase deficiency) was diagnosed with bilateral vulval carcinomata. She was referred to another unit with serum sodium of 121 mmol/l, potassium 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l). A short synacthen test performed in the evening demonstrated a baseline cortisol of 4.6 mmol/l and 17-hydroxyprogesterone of 714 nmol/l (0–14 nmol/l).

Despite surgical resection, radiotherapy and chemotherapy, the vulval carcinomata recurred. She was then admitted to our hospital unwell with BP 79/50 mmHg. Blood tests showed sodium 119 mmol/l, potassium 5.8 mmol/l and 9am cortisol of 1357 nmol/l. Further 9am investigations showed a repeat cortisol of 1350 nmol/l, testosterone 13.6 nmol/l (0.1–1.5 nmol/l), FSH 2.9 IU/l, LH <0.2 IU/l, 17-hydroxyprogesterone 470 nmol/l (0–14 nmol/l), ACTH 371 ng/l (0–40 ng/l), and dehydroepiandrosterone 2.1 μmol/l (0–4.6 μmol/l). 24 h urine collections for free cortisol were raised at 378 nmol/day and 483 nmol/day (0–260 nmol/day).

Clinically there was evidence of hypoadrenalism but there was biochemical evidence of hypercortisolaemia. MRI pituitary to rule out Nelson’s syndrome was normal. The possibility of cortisol assay interference was considered accounting for the elevated serum and urine cortisol. Her 24 h urine sample was then analysed at two national reference laboratories who confirmed steroid precursor assay interference accounting for the markedly elevated ‘cortisol’ measurements. She unfortunately succumbed to her carcinoma (on steroid replacement therapy).

Discussion

This case demonstrates how tests can be misleading. The clinical picture indicated a hypoadrenal state (hypoamena/hyperkalaemia/hypotension) but the measured cortisol was falsely measuring. This serves us to remember that a good clinical assessment is crucial.
P50
A clinico-microbiological study of infected diabetic foot ulcers
Pinaki Dutta, M N Parvez, P Ray, L Kuman, Anil Bhansali, K Mahesh & N Khandelwal
Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Background
According to western literature aerobic Gram-positive cocci are the predominant microorganisms that are isolated from diabetic foot ulcers. In contrast, whatever limited data is available from India Gram-negative aerobic bacteria were most frequently isolated.

Aims and objectives
To know the clinical and microbiological profile of patients with infected diabetic foot ulcers.

Patients and methods

Introduction
Hyponatraemia is not uncommon in primary care and its management can be complex. It is vital that initial assessment is carried out properly as mismanagement can have serious consequences.

Aim
To investigate general practitioners’ views and perceptions on the management of hyponatraemia encountered in primary care.

Method
Local general practitioners were surveyed with a questionnaire via email and at local postgraduate meetings. Questions were themed around the following areas:

i) the management of hyponatraemia
ii) confidence in managing hyponatraemia
iii) experience managing hyponatraemia including long-term complications
iv) training needs

Results
A total of 65 general practitioners returned completed questionnaires. The median (range) suggested serum sodium concentration (mmol/l) for arranging further investigations was 130 (118–135), for routine referral to secondary care was 125 (105–130), and urgent referral 120 (100–128). When asked about the importance of a serum sodium of 125–130, 63% of respondents replied ‘moderately’ important, 25% very important and 12% little importance. When asked what further tests would be considered at this level of hyponatraemia, the majority (65%) would repeat serum electrolytes. Only 9% mentioned urinary sodium and paired plasma/urine osmolality.

The commonest cause of hyponatraemia that respondents had encountered was drug-related (78%). Most respondents felt they had not received sufficient training (85%) and lacked confidence in the initial assessment of hyponatraemia (64%). Those qualified >20 years felt less confident and were less likely to suggest further tests as recommended by specialists. 77% of respondents would like a management protocol or algorithm and 60% were keen to attend a local educational event on hyponatraemia.

Conclusion
In our area, many general practitioners lack confidence in the management of hyponatraemia and practice varies widely. There is a large unmet educational need in this area and future studies will look at how best this can be addressed.

P51
A calcium sensing receptor mutation associated with hypercalcaemia and recurrent pancreatitis
Lesley Hall1, Dairena Gaffney1, Joanne Ramsay1, Andrew Gallagher1 & John Hinnie1
1Diabetes Centre, New Victoria Hospital, Glasgow, UK; 2Department of Biochemistry, Glasgow Royal Infirmary, Glasgow, UK.

A 29-year-old man of South Asian descent presented with pancreatitis. Adjusted calcium was 3.10 mmol/l and PTH 9.8 µmol/l. Of note was that his parents were first cousins, and a cousin had undergone parathyroidectomy for hypercalcaemia. Pituitary, adrenal and thyroid function, calcitonin, prolactin, phosphate, magnesium, alkaline phosphatase and urinary catecholamines were all normal. 25-Hydroxyvitamin D was 20 mmol/l (15–100). The patient was therefore commenced on ergocalciferol, 0.25 mg daily. Urinary calcium excretion was low at 0.5 mmol/24 h (2.50–7.50). Parathyroid imaging was normal. Calcium sensing receptor (CASR) sequencing showed a C>T mutation 10 bps before the CASR start codon. This created an ATG sequence which could be a premature out-of-frame start codon. The patient was homozgyous for this mutation while both parents and all 5 siblings were heterozygous and had normal serum calcium concentrations. The patient went on to have several further episodes of pancreatitis associated with hypercalcaemia, following which he was commenced on cinacalcet. Serum calcium gradually decreased and he remained well for 18 months. Unfortunately thereafter he had further episodes of pancreatitis and hypercalcaemia, suggesting non-compliance with cinacalcet or loss of drug effect.

Three years after initial presentation he underwent total parathyroidectomy. Parathyroid histology was normal. Postoperatively, he was commenced calcium carbonate and alfalcalcidol. He went on to have a further episode of pancreatitis with hypercalcaemia, but with a PTH of only 0.8 µmol/l. Following this alfalcaldol dose was reduced and calcium carbonate discontinued. Since then he has not experienced further hypercalcaemia. However, he continues to have intermittent pancreatitis which is felt to be due to pancreatic duct stenosis. We discuss the possibility that the mutation detected leads to a lack of functioning receptor and thus hypercalcaemia.
He was commenced on anti-thyroid medications and is scheduled for Fistulography.

### P53

**Echocardiogram in prolactinoma patients taking ergot derived dopamine agonists**

Auditi Naziat$^1$ & Mohgah Elsheikh$^2$

$^1$Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK; $^2$Centre for Endocrinology and Diabetes, Royal Berkshire Hospital, Reading, UK.

**Background**
The use of high dose Ergot derived dopamine agonists in patients with Parkinson’s disease has been associated with an increased risk of cardiac valvular fibrosis. The UK Medicines and Health products Regulatory Agency (MHRA) currently advises baseline Echocardiogram within 3–6 months and follow up Echocardiogram at 6–12 monthly intervals in all patients taking ergot derived dopamine agonists. However, the risk of cardiac valvulopathy with lower doses used to treat prolactinomas remains unclear.

The aims of this study were to audit our adherence to the MHRA guidance in patients treated with ergot derived dopamine agonists for prolactinoma and to determine the prevalence of valvular heart disease in this cohort of patients.

**Methods**
Retrospective casenote review ($n=48$) of patients with prolactinoma on Cabergoline ($n=42$) or Bromocriptine ($n=6$) attending Endocrine clinic. Thirty-six were already on treatment prior to the publication of MHRA drug safety advice. Seventeen patients were on treatment for $>5$ years, 26 under 5 years, four $<6$ months and one of unknown duration.

The median Cabergoline dose was 500 mg/week and Bromocriptine 1.88 mg/day.

**Results**
Thirty-five patients (72.9%) had a baseline echocardiogram of whom 22 (62.8%) had a normal result and 13 (37.50%) mild/trivial TR, AR or MR. Thirteen (6) attending Endocrine clinic.

None had follow up echocardiograms; 26 were not due (<12 months of baseline ECHO), one discontinued cabergoline and no reason documented in 7.

**Conclusion**
83.33% patients diagnosed after the MHRA advice had baseline echocardiograms within 6 months of treatment. Sixty-six percent patients on treatment predated MHRA advice had an echocardiogram as soon as the advice was published. In contrast to treatment with high dose cabergoline in patients with Parkinson’s disease no significant cardiac valvular dysfunction was found on echocardiogram in patients with prolactinoma treated with low-dose dopamine agonists.

### P54

**Management of adrenal incidentaloma: are we getting it right?**

Lauren Price, Srinu Munigoti & Aled Rees

Cardiff University, Cardiff, UK.

**Background**
Adrenal incidentalomas (AI) are a common radiological finding (estimated prevalence 4%). Guidelines from the American Association of Clinical Endocrinologists (AACE) and the American Association of Endocrine Surgeons (AAES) recommend radiological evaluation at 3–6 months then annually for 1–2 years, and hormonal evaluation by measurement of plasma aldosterone/renin (ARR) activity, 24 h catecholamines/metanephrines (CATS), and 1 mg overnight dexamethasone suppression test (ONDST).

**Aims**
To compare the prevalence of AI reported on abdominal CT scans at our institution with the literature and to identify how many patients were referred for endocrine review. To compare our management of AI with the AACE/AAES guidelines.

### P55

**Hypoadrenalism then adrenal haemorrhage as manifestation of lymphoma relapse after 3.5 years**

Mansour Seidahmad, Panagiota Anna Chousou, Firas Haddadin, Emad George & Adrian Jennings

Diabetes and Endocrinology Centre, Queen Elizabeth Hospital, King’s Lynn, Norfolk, UK.

Hypoadrenalism and bilateral adrenal haemorrhage are rare manifestations of lymphoma. We present a case of diffuse large B cell non-Hodgkins lymphoma (NHL) in whom the main manifestations of relapse included hypoadrenalism and then bilateral adrenal haemorrhage.

A 75-year-old male presented with a 2-week history of severe left sided abdominal pain. He was known to have NHL predominantly involving the right maxillary sinus, which had been treated with chemotherapy and had been in remission for 3.5 years. He also had a history of type 2 diabetes mellitus, adenocarcinoma of the prostate and pulmonary embolism treated with warfarin.

He had been admitted 3 months previously under another team with transient leg weakness and had been noted to be hypotensive. A short synchiran test had shown a suboptimal response (peak cortisol 434 nmol/l) so he received hydrocortisone replacement.

Examination showed he was afebrile, heart rate 82/min and blood pressure 135/62 mmHg. There was left sided abdominal tenderness.

His initial blood results showed haemoglobin 7.2 g/dl and a prolonged INR of 9.

An urgent CT scan showed massive bilateral adrenal haemorrhage with mild splenomegaly and some enlarged retroperitoneal lymph nodes.

His anti coagulation was reversed, he was transfused and treated with i.v. hydrocortisone. He responded well but then developed recurrent febrile episodes.

These responded to antibiotics initially, but despite aggressive treatment, the patient’s condition deteriorated and he died 52 days after admission. Post mortem examination revealed lymphoma and haemorrhage in both adrenal glands. There was also recurrent lymphoma in an intra-abdominal lymph node. No other factors contributing to his death were identified.

Adrenal imaging is warranted when hypoadrenalism presents in a patient with lymphoma in remission as it may indicate relapse. When adrenal haemorrhage occurs in patients with lymphoma adrenal lymphoma should be considered.
Methods
Patients referred to bariatric surgery clinic were investigated for hypothyroidism (TSH, T4), Cushing’s disease (2 mg-overnight dexamethasone suppression test), acromegaly (IGF1) and Vitamin D deficiency (PTH, Ca and Vitamin D) based on clinical suspicion. A retrospective observational analysis was conducted to analyse the prevalence of endocrine disorders and distribution of comorbidities.

Results
Demographics: n = 159 patients; mean age: 42.6 years (17–67); females: 80%; mean BMI 49.5 kg/m² (35–73).
Distribution of comorbidities: Clinical Diabetes mellitus: 30.2%, Hypertension: 37.7%, Dyslipidemia: 34%, Severe arthritis: 39%, Obstructive sleep apnoea: 6%.
Cushing’s syndrome: n = 85; None of the patients had documented cushingoid morphology. Apart from one patient who had unexpressed cortisol (68 mmol/l; urinary free cortisol normal – not investigated further), all the others were negative.
Thyroid status: n = 159; 13% were known hypothyroidism on replacement; 33% of them were inadequately replaced. 1.4% had sub-clinical hypothyroidism not being treated so far.
Acromegaly: n = 52; All had normal age-related IGF1 levels.
Bone: PTH: n = 105; 61% had high PTH (≥6.4 pmol/l). Serum calcium was within normal range in all patients. PTH values correlated positively to BMI (r = 0.1). Vitamin D: n = 81; 16.1% deficient (< 10 μg/l); 60.5% were insufficient (11–30 μg/l); 23.4% were Vitamin D replete (> 30 μg/l). Vitamin D correlated negatively to BMI (r = −0.2).

Conclusion
A vast majority of patients with morbid obesity, who are referred for bariatric surgery, do not have an endocrine aetiology. Vitamin D deficiency or insufficiency is present in a high proportion of this cohort (77%) and hence should be treated prior to surgery and reassessed post-operatively. Thyroxine treatment should be optimized in patients with prior hypothyroidism. Screening for Cushing’s syndrome or acromegaly need not be performed unless clinically indicated.

P57
Effective use of Cinacalcet in tertiary hyperparathyroidism in a patient with hypophosphataemic rickets
Madeleine Banastwrou1, Helen Moore1, Niru Goenka1, David Ewins1, Anindya Banerjee2 & Frank Joseph1
1Department of Diabetes and Endocrinology, Countess of Chester Hospital, Chester, UK; 2Department of Nephrology, Countess of Chester Hospital, Chester, UK.

A 22-year-old woman with hypophosphataemic rickets was diagnosed at age four when she presented with short stature and valgus deformity of the lower limbs. Biochemical testing, genetic screening and radiological investigation of her family showed no abnormality and it was concluded that she had a de novo mutation. She was treated with 1α-calcidol and phosphate Sandoz with regularly monitored biochemistry. She had poor adherence to her medication and her phosphate levels fluctuated. Her valgus deformity showed little improvement and she required stapling of her left distal medial epiphyseal plate at age 15 years.

At age 14 she had been diagnosed with secondary hyperparathyroidism with calcium 2.38 mmol/l, phosphate 0.91 mmol/l and parathyroid hormone (PTH) 24.71 pmol/l. Her renal function was normal. The need to adhere to 1α-calcidol was reiterates, but her PTH remained elevated. At age 18 she developed hypercalcemia with calcium 3.19 mmol/l, phosphate 1.61 mmol/l and PTH 10.3 pmol/l and her 1α-calcidol was gradually reduced to 500 μg once a week. Despite this her calcium remained elevated at 2.85 mmol/l with a progressively increasing PTH (65.4 pmol/l) confirming a diagnosis of tertiary hyperparathyroidism. She had a sestamibi scan which identified bilateral lower pole parathyroid adenomas, for which she declined surgery and was therefore treated with cinacalcet, starting at 30 mg daily and increased to 90 mg daily in divided doses. The calcium and PTH decreased to 2.58 mmol/l and 23.4 pmol/l respectively over a period of 8 months. β-CTX a marker of bone resorption was normal at 0.3 μg/l (NR 0.1–0.5).

Although cinacalcet is licenced for treatment of tertiary hyperparathyroidism, this case demonstrates the effective use of cinacalcet in lowering PTH and calcium in tertiary hyperparathyroidism in hypophosphataemic rickets. The normal β-CTX concentration would also suggest that there is benefit at the target organ, bone, by maintaining normal bone resorption which would otherwise be high in hyperparathyroidism.

P58
Long-term single centre outcome of phaeochromocytoma/paraganglioma
Huda Al-Kutubi1, Joanne Greenwood2, Maneesh Udiawar2, Atul Kalhan2, David Scott-Coombes2 & Aled Rees1
1Cardiff University, Cardiff, UK; 2Cardiff and Vale University Health Board, Cardiff, UK.

Background
Phaeochromocytomas (PHAE0s) and paragangliomas (PGLs) are rare catecholamine producing tumours which are potentially lethal if left untreated and may be associated with a wide variety of complications. Optimum management demands multidisciplinary input from endocrinologists, biochemists, geneticists and endocrine surgeons.

Objective
A retrospective audit into the management of PHAE0s/PGLs at our institution against the 2005 recommendations made at the 1st International Symposium.

Methods
Data were collected on all patients who had presented to the endocrine department with a diagnosis of PHAE0 or PGL over a 12 year period (1997–2009). This was cross-checked with a biochemistry database capturing all patients with raised urinary catecholamines and/or metadrenalin.

Results
Fifty-nine patients with a confirmed diagnosis of PHAE0 (48 unilateral, 6 bilateral) or PGL (5) were identified. Biochemistry (post-2003: 24 h urine for fractionated metadrenalin plus catecholamines) demonstrated elevation of at least one fractionated metadrenaline in all subjects at presentation. Localisation studies included a combination of CT (60%), MRI (42%) and 131I-MIBG (64%). 30% of patients were found to harbour a mutation in a susceptibility gene (8% VHL, 8% MEN2A, 7% NF1, 7% SDHB/D); of the remaining 41 subjects, genetic testing was indicated in 37% but only undertaken in half of these. All patients were treated surgically (84% laparoscopic since 2004) with low morbidity and mortality (<2%); preoperative adrenergic blockade was undertaken in all.

Conclusions
Biochemical screening using fractionated urinary metadrenalin provides a high diagnostic sensitivity for PHAE0 and PGL. Functional imaging is used selectively in our institution but surgical outcome is good. Genetic testing reveals a relatively high rate of inherited endocrine tumour syndromes but is under-requested in a small minority of cases.

P59
An analysis of false positive urinary catecholamine and metabolite results in a tertiary endocrine centre
Huda Al-Kutubi1, Joanne Greenwood2, Atul Kalhan2, Maneesh Udiawar2 & Aled Rees1
1Cardiff University, Cardiff, UK; 2Cardiff and Vale University Health Board, Cardiff, UK.

Background
The screening investigation of choice for phaeochromocytomas (PHAE0) and paragangliomas (PGL) in the UK is usually a 24 h urine collection for fractionated metadrenaline and/or free catecholamines. These assays have high diagnostic sensitivity (approaching 98%) but lower specificity.

Aim
To review causes of false positive (FP) catecholamine and metabolite results in our centre over a 12-year period.

Methods
Data were collected on all patients who presented to our endocrine department with a histologically confirmed diagnosis of PHAE0 or PGL between 1997 and 2009. This was compared with a biochemistry database capturing all patients with raised urinary catecholamines and/or metadrenalines. A test was deemed to be FP when repeat biochemistry and/or radiology (CT/MRI/MIBG) was consistently negative. Clonidine-suppression tests were not performed.

Results
Fifty-nine patients with PHAE0 or PGL were identified and compared with 57 FP results in 47 patients (elevated metadrenaline n = 11, normetadrenaline n = 11, adrenaline n = 7, noradrenaline n = 17, dopamine n = 11). Compared with PHAE0/PGL, FP typically showed only moderately elevated catecholamines/metadrenalines: results were no higher than 1.75 (adrenaline), 1.8 (noradrenaline), 1.8 (dopamine), 2.9 (metadrenaline) or 3.1 (normetadrenaline)× upper limit of normal.
P60 Juxta-adrenal Schwannoma presenting as ‘Giant’ adrenal adenoma

Smitha Amruchetty, Peter Donaldson, Charlotte Etheridge, Ian Driver & Craig Parkinson
Ipswich Hospital NHS Trust, Ipswich, UK.

A 65-year-old female, with a 4 month history of left upper quadrant discomfort, was identified as having a multi-lobulated para-renal ‘cyst’ on ultrasound scanning. CT identified a 13x11x10 cm heterogeneous mass arising from the left adrenal. An enlarged ill-defined left retro-crusal ‘lymph node’ was also noted. There was no history of weight loss. Past medical history was unremarkable. She was not on any medication. Examination was unremarkable apart from a BP of 181/102 mmHg.

Pre-operative endocrine assessment: normal electrolytes, 24 h UFC, urinary metanephrines (2x), testosterone, androstenedione, and DHEAS. Post 48 h low dose dexamethasone suppression test (LDDST) serum cortisol = 55 nmol/l. Based on size criteria, lymph node involvement and apparent low grade Cushing’s syndrome, the patient was informed that the mass most likely represented an adrenal adenocarcinoma and she was listed for surgery.

Under corticosteroid cover, left radical adrenalectomy, nephrectomy, and excision of the retro-crusal lesion was undertaken. She was discharged on replacement hydrocortisone. After a normal short synacthen test hydrocortisone was withdrawn. A repeat LDDST was normal.

The solid cystic encapsulated mass weighed 670 g, measured 130x120 x 110 mm and was histologically separate from the left adrenal. Composed of spindle cells (positive for S-100 protein and Vimentin but negative for Actin) with Verocay bodies (Antoni A areas) and less cellular Antoni B areas, there was no evidence of cellular pleomorphism or mitotic figures. The retro-crusal mass was composed of similar spindle cells.

Pathological diagnosis was a benign ‘giant’ juxta-adrenal schwannoma and retro-crusal schwannoma.

Retropertioneal and juxta-adrenal schwannomas are extremely rare and may appear pre-operatively as adrenal tumours. Given their size (>3 cm) they may be confused with adrenocortical carcinomas and phaeochromocytomas, particularly given that some case reports highlight uptake of MiBG by these lesions. The failure of cortisol suppression following dexamethasone administration was, in retrospect, spurious.

P61 Life-threatening adverse reaction following pituitary MRI

Katerina Achilles, Tehmina Irani & James Ahlquist
Southend Hospital, Westcliff on Sea, Essex, UK.

Pituitary MRI is widely used in endocrine practice, and is regarded as entirely safe. We report here a life-threatening outcome from a routine pituitary MRI scan. A 23-year-old female with a 3-year history of microprolactinoma confirmed by MRI underwent a routine repeat MRI scan with gadolinium. During injection of Gadovist she experienced minimal chest tightness which rapidly resolved. Four hours after the injection she rapidly became very breathless. On admission to hospital she was shocked, profoundly breathless, with cyanosis, hypotension and marked hypoxia (HR 162 bpm, BP 72/50 mmHg, PaO2 7 kPa despite FiO2 60%).

There were diffuse crepitations throughout both lung fields and no signs of cardiac disease or angioedema. CXR showed bilateral perilhveolar alveolar shadowing, indicating pulmonary oedema (ARDS). She was treated with high-flow oxygen, adrenaline, hydrocortisone, chlorpheniramine and furosemide. She remained critically ill and was admitted to ITU, where she required inotropes and CPAP non-invasive ventilation for persistent acute respiratory failure. Echocardiogram confirmed normal cardiac function. She made a rapid recovery and was discharged home well 2 days later.

She subsequently recalled that she had felt slightly unwell after her first MRI scan 3 years earlier.

Acute lung injury has not previously been reported after gadolinium administration. Gadolinium-induced serious adverse reactions are extremely rare (1–3 per million administered doses). Gadovist is a modern contrast agent regarded as having a very low potential for anaphylactoid reactions; it includes a macrocyclic chelate which is thought to give less risk of gadolinium toxicity than older agents with a linear chelate such as Omniscan. However, macrocyclic gadolinium agents may be associated with a higher frequency of allergic reactions.

Pituitary disease is rarely fatal. Endocrinologists should be aware that pituitary MRI carries a small risk of iatrogenic adverse reaction which may be life-threatening.

P62 Audit on colonoscopy screening in acromegaly patients in a tertiary endocrine unit

Daniel Kannappan, Sami Kenz & Tara Kearney
Salford Royal Hospital, Manchester, UK.

Aim
To find out all Acromegalic patients above the age of 40 years had their colonoscopic screening or not.

Reason for colonoscopy screening
There is 13 to 14 fold increased risk of colorectal cancer in Acromegaly patients and 2.5 fold increase in mortality from Colonic cancer.

Standards
i) Patients with acromegaly should be offered regular colonoscopy from the age of 40 years.

ii) The Frequency of repeat colonoscopy depends on the findings at the original screening and the activity of the underlying acromegaly.

iii) Patients with adenoma or increased IgF1 needs screening at 3 yearly intervals.

iv) Negative or hyperplastic polyp in the first colonoscopy needs screening at 5 yearly intervals.

v) Total colonoscopy is required rather than sigmoidoscopy as 25% of adenomas and 50% of carcinomas occur in ascending and transverse colon.

Data analysis
i) Total number of Acromegaly patients who had treatment in our centre was 89.

ii) Out of this 53 patients were under follow up in our hospital and 36 patients were referred back to their original hospital.

iii) 25 patients (47%) had colonoscopy or virtual colonoscopy and for 5 patients no information was available.

iv) 18 patients had normal screening, 4 patients had hyperplastic polyp and the remaining 3 patients had adenoma, ulcer and Crohns colitis respectively.

v) 23 patients did not have colonoscopy. Out of which 8 patients were less than 40 years and screening is not indicated.

vi) Remaining 15 patients (30%) did not have colonoscopy screening.

Conclusion
The above audit showed nearly one third of our Acromegaly patients did not have colonoscopy screening. This audit has changed our clinical practice and going to be re audited in 12 months.

P63 Adrenal haemorrhage associated with therapeutic Clexane and subtherapeutic warfarin

Panagiota Anna Chousou, Mansour Seidahmad, Firas Haddadin & Adrian Jennings
Diabetes and Endocrinology Centre, Queen Elizabeth Hospital, King’s Lynn, Norfolk, UK.

Adrenal haemorrhage is rarely associated with anticoagulation according to a large American series. We present 2 cases in whom there was no evidence of over anticoagulation, both of whom developed a degree of hypoadrenalism.
P64
A case of SIADH and hyponatraemia treated successfully with Tolvaptan
Kamal Abougilla, Nicola Robinson & Emily Curran
UHND, Durham, UK.

Hyponatraemia complicates 1% of hospital admissions and can be associated with serious CNS effects. We report a case of an 84-year-old woman with longstanding hyponatraemia resulting in several hospital admissions because of acute confusion due to severe hypoosmolality. This case emphasizes the need to consider selective V2-receptor antagonist (Tolvaptan) as a potential therapy for hyponatraemia secondary to the syndrome of inappropriate antidiuretic hormone (SIADH).

Case report
A 46-year-old female presented with acute confusion, agitation, and generally unwell. Past medical history of significance included hypothyroidism and diabetes mellitus and she was not taking any medication to contribute to her confusion. Physical examination, Chest X-ray and CT of the head were unremarkable. Serum sodium was 118 mmol/l, serum osmolality 267 mOsm/kg, urine osmolality 362 mOsm/kg, and urine sodium 70 mmol/l. Thyroid function test and serum cortisol results were both normal. Looking back to her notes we notes that the serum sodium results were low most of the time for the last few 18 months. Cognitive testing revealed time disorientation and poor concentration. All the investigations confirm the diagnosis of SIADH as a cause of hyponatraemia and the cause of SIADH was idiopathic. She treated initially with restrict fluid restriction for 12 days but here sodium remained low 122 mmol/l. The decision was then made to start her on Tolvaptan and the fluid restriction was stopped. She remained on Tolvaptan for 8 days until her was 132 mmol/l. She was discharged two days later with sodium of 134 mmol/l.

Conclusion
This case highlights the importance of the use of Tolvaptan to correct sodium gradually and its potential for reducing the length of stay in hospital.

P65
Proton pump inhibitor (PPI) therapy in patients admitted to a diabetes and endocrine medical ward: are there clear indications?
Luke Teo, Michelle Mok, Andrew Macnair, Prakashmini Nunkoo, Albert Lim, Siobhan Mutthia, Margaret Warlow, Christian Dipper & Richard Quinton
Royal Victoria Infirmary, Newcastle Upon Tyne, UK.

Introduction
Proton pump inhibitors (PPIs) were until very recently perceived to be safe, effective and inexpensive. As a result they are widely prescribed empirically, beyond their core indications. However dose-dependent adverse reactions increasingly reported include diarrhea, resistant hypomagnesaemia/hypocalcaemia and interstitial nephritis. Moreover, they are associated with increased rates of vertebral and wrist fractures, and increased C. difficile carriage rate.

Aims
To ascertain the extent and appropriateness of PPI prescribing in patients admitted to the Diabetes and Endocrine medical base ward in the Royal Victoria Infirmary (RVI).

Methods
All PPIs prescribed should have a clear indication, duration for treatment and/or date of review.

Results
Over an 8 week period, 105 consecutive admissions to Ward 31, RVI were evaluated for i) dose of PPI prescribed (‘low-dose’, ‘standard dose’ or ‘high dose’); ii) identifiable indication from patient history, admissions proforma, pharmacist history, GP referral letters and/or electronic records.

Synacthen testing showed a suboptimal response (peak serum cortisol 524 nmol/l). Eleven months after discharge a repeat CT scan was normal apart from minor thickening in the left adrenal gland.

Conclusion
Nineteen percent of new patients admitted to a general medical ward were taking a PPI for no clear indication. When a drug is perceived to be safe, effective and inexpensive, documentation tends to be sparse. Although PPIs cause hypomagnesaemia/hypocalcaemia in only a tiny proportion of patients, they are so widely prescribed that they possibly constitute the most common cause of hypomagnesaemia/hypocalcaemia among hospital inpatients.

Suggests
PPIs should only be used for their core indications. When a PPI is started, expected duration of therapy and date of review should ideally be established at the outset. Clear record keeping by GPs and hospital doctors is essential. If no indication is found in secondary care, the PPI dose should be reduced at least and the primary care physician informed.

P66
Pituitary thyroid hormone resistance (PTHR)
Ahmed El-Laboudi & Steve Orme
Leeds Teaching Hospitals NHS Trust, Leeds, UK.

A 32-year-old lady was referred to our centre with thyrotoxicosis and elevated FT4 and TSH levels. She was already on carbimazole. Interestingly, her symptoms started at childhood. She was nicknamed ‘shaky’ by her school friends because of her tremors. There was no family history of thyroid disease.

She was clinically and biochemically thyrotoxic with FT4 of 12.4–38.8 pmol/l and TSH of 7.24–38.8 mIU/l. After excluding assay interference as a possibility, Investigations revealed a normal TSH subunit/TSH molar ratio, an appropriate rise in TSH during TRH test and no evidence of a pituitary adenoma on MRI. This suggested a diagnosis of pituitary thyroid hormone resistance (PTHR). However, sequencing thyroid hormone receptor β (THR-β) gene did not identify any abnormality.

Following a TSH day curve to assess response to cabergoline and octreotide, carbimazole was switched to carbimazole which only resulted in partial clinical and biochemical improvement. She underwent further assessment at Addenbrooke’s Hospital. Negative genetic testing raised doubts about initial diagnosis. Re-sequencing THR-β gene and repeat MRI scan again did not identify any abnormality. Also, measurement of BMR, sleeping heart rate, SHBG and BMD to assess peripheral thyroid hormone actions confirmed the initial diagnosis.

Her thyrotoxicosis improved after adding triiodo-thyroacetic acid (TRAIC) to her treatment regimen. However, following MHRA guidance, a routine echocardiogram revealed severe MR. Therefore, cabergoline was switched to quinagolide following a repeat TSH day curve.

She underwent successful cardiac surgery. Currently, she is clinically and biochemically euthyroid and in addition to nadolol is taking TRAIC 700 µg BD and quinagolide 150 mg daily.

This case that revealed symptoms of thyrotoxicosis since childhood (nickname ‘shaky’) highlights important learning points including: diagnostic approach to a patient with TSH-induced thyrotoxicosis, value of TSH day curve, use of TRAIC and the relationship between carbimazole and fibrotic valvular heart disease.
A 76-year-old man with history of oesophageal carcinoma was successfully treated by surgery and radiotherapy 4 years previously. He presented with anorexia and 4 stone weight loss associated with abdominal discomfort. He was not on any medication. On examination blood pressure 100/60, no peripheral oedema, systemic examination otherwise was unremarkable. Soon after admission, he developed a generalized tonic-clonic seizure requiring a Lorazepam and Phentoin infusion to control it in critical care setting. He became hypotensive with blood pressure of 93/49. Subsequent investigations revealed significant hyponatraemia of 118 mmol/l, severe hypoglycaemia, blood glucose 1.8 mmol/l, potassium 4.4 mmol/l and serum bicarbonate 21 mmol/l. The rest of blood tests including: full blood count, liver functions, corrected calcium, urea and creatinine were all normal.

As an urgent CT head revealed no metastasis and his clinical and biochemical indices pointed towards Addisonian crisis therefore the patient was commenced on hydrocortisone, normal saline and the hypoglycaemia was treated with glucagon and dextrose. On the following day there was no further seizure activity, biochemical profile normalised and patient started mobilising independently. Short syncheton test showed a flat cortisol response consistent with adrenal failure. Furthermore, abdominal CT scan revealed bilateral adrenal enlargement with heterogeneous appearance suggestive of metastatic deposits. He was seen by the oncologist and allowed home on hydrocortisone and fludrocortisone.

We report a case of status epilepticus due to a combination of severe hypoglycaemia and hypoglycaemia as a presentation of an undiagnosed Addison’s disease in a patient with underlying neoplasm. Hyponatraemia and hypoglycaemia, both of which could be features of malignancy as in SIADH and hypoglycaemia with malignancy. This case highlights the importance of high index of suspicion in relevant settings to make a diagnosis of Addison’s disease.

A 31-year-old male presented to A&E with confusion, lethargy, polyuria and blurred vision. The previous month he had been diagnosed with diabetes and started on metformin by his GP. On examination he was cachectic (BMI 17 kg/m²), dehydrated and hypotensive (88/56). Baseline investigations revealed a metabolic acidosis (pH 7.30), with capillary glucose 20 mmol/l and moderate ketonuria. Treatment for diabetic ketoacidosis was commenced. Further results revealed he was hyponatraemic (sodium 111 mmol/l). Over the next 48 h, his condition improved significantly with insulin sliding scale (converted to basal bolus regime after 24 h) and i.v. fluids, however his sodium did not fully correct and reached a plateau of 127 mmol/l by day 4 post admission.

Further investigations revealed a 0900 h cortisol of 521 mmol/l however his ACTH was markedly elevated at 329 ng/l. The diagnosis of Addison's was confirmed on a short synacthen test (time 0 min cortisol 386 mmol/l, 30 min 397 mmol/l and 60 min 378 mmol/l). He was started on hydrocortisone and fludrocortisone after which his sodium corrected to normal.

Co-presenation of type 1 diabetes and adrenal insufficiency is uncommon but suggests an underlying diagnosis of type 2 polyglandular autoimmune syndrome. Further tests revealed he was positive for islet cell and anti-GAD antibodies, but not adrenal cortex antibodies. Thyroid and gonadal function was normal, as were his vitamin B12 levels. The hyponatraemia was likely contributed to by hypoglycaemia, an appropriate ADH response to hypotension and hypocortisolaemia.

Conclusions

Though the prevalence of type 2 diabetes is on the increase, practitioners should be alert to the possibility of late-onset autoimmune type 1 diabetes, particularly in those of a lean phenotype. Hyponatraemia can be multifactorial in origin, and an incompletely corrected level should prompt further investigation. A normal cortisol in the context of acute illness may be misleading and an ACTH is of value in this setting.

A 30-year-old female reported a 2-year history of excessive sweating / flushing affecting the right hand side of the face and scalp along with the right arm. These symptoms were generally related to exercise and demonstrated a sharp midline demarcation, being present only on the right hand side.

Past medical history included hypothyroidism secondary to Hashimoto’s Thyroiditis. She was euthyroid on levothyroxine. She also reported unequal pupils since age 12.

On examination, she was normotensive with no postural change in blood pressure. A tachycardia of 132 bpm was confirmed on ECG. She had tonic pupils, the right larger than left, with absent knee and ankle reflexes. No goitre or thyroid nodules were palpable.

Urine collections were negative for metanephrines and 5-HIAA. MRI cervical spine showed normal cervical spine with no surrounding mass lesions or thyroid abnormality.

A diagnosis of Ross’ syndrome, with left sided hypothalamic and compensatory hyperhidrosis on the right, was established. Ross’ syndrome is the triad of tonic pupils, hyporeflexia and segmental anhidrosis with compensatory hyperhidrosis. This encompasses Holmes Adie syndrome – tonic pupils and hyporeflexia. The cause is unknown but is thought to result from generalised injury to peripheral autonomic and dorsal root ganglia. Proximally is unclear and although generally benign, progression of cardiovascular autonomic dysfunction and widespread autonomic involvement is described in 28-40% of patients. Females are more commonly affected with average age on onset of 32. Presentation may be with orthostatic hypotension, due to afferent baroreceptor failure.

The diagnosis can be confirmed by autonomic testing and pupillography. No specific treatment is possible for the hyperhidrosis, but this may respond to cervical or thoracic sympathectomy.

Ross’ syndrome is distinguished from Horner’s syndrome where a Horner’s complex is usually present and abnormalities of the inferior thyroid artery are often found.
Does the glucagon challenge test add to the utility of a 72 h fast for spontaneous hypoglycaemia?  
Anupam Brahma, Steven Romans, Sondra Gorick, K Sw M Yiint & F M Swords  
Norfolk and Norwich University NHS Foundation Trust, Norwich, UK.

Supervised 72 h fast is historically the gold standard screen for spontaneous hypoglycaemia. Consensus guidelines published in 2009 suggested that β-hydroxybutyrate levels be measured every 6 h, and the glucose response to an i.v. injection of glucagon be assessed at the time of determined hypoglycaemia, or at the end of the 72 h fast. We incorporated these recommendations into our protocol, and here examine the results of the first 10 cases.

A rise in plasma β-hydroxybutyrate levels ≥ 2.7 mmol/l may be interpreted as a surrogate for hypoinsulininaemia. During the 72 h fast period, all 10 patients in whom hypoglycaemia was ultimately excluded exhibited a rise in plasma β-hydroxybutyrate levels. By the end of the fast, 50% had plasma β-hydroxybutyrate levels ≥ 2.7 mmol/l indicating that their fasts could have been terminated earlier. Twenty percent had levels of 2.6 mmol/l and 30% were < 2 mmol/l. We interpret the rise to confirm adherence to the fast, but patients in whom levels remain < 2.7 mmol/l require the fast to continue.

This entire cohort also underwent glucagon stimulation at the end of the negative fast. No serious adverse reactions were reported, though nausea and flushing were common, and the challenge also prolonged the total test period. In the presence of hypoglycaemia, a rise in glucose by more than 1.4 mmol/l (25 mg/dl) in response to glucagon is consistent with insulin or IGF mediated hypoglycaemia. A smaller rise is found in normal subjects, and hypoglycaemia of other mechanisms. Hundred percent patients demonstrated a rise < 1.4 mmol/l in this study.

We suggest that serial monitoring of plasma β-hydroxybutyrate levels during a fast may allow premature termination of the fast if levels > 2.7 mmol/l, and confirms adherence to the fast when spontaneous hypoglycaemia is not confirmed. However, glucagon challenge at the end of a negative fast adds little to the interpretation, and is unnecessary.

Introduction of day-case thyroid and parathyroid surgery has allowed achievement of the shortest length-of-stay in the United Kingdom
Rajeev Parameswaran, Gregory Sadler & Radu Mihai  
John Radcliffe Hospital, Oxford, UK.

Background  
Length-of-stay (LOS) after various surgical procedures varies greatly between centres and countries. In the US there is increasing tendency towards day-case thyroid and parathyroid surgery but their model of care cannot be easily extrapolated. In the UK the average LOS for such operations is 3–4 days.

Methods  

Results  
During January 2000–June 2010 a total of 1934 patients underwent thyroid (n = 1264) and parathyroid (n = 670) surgery. Gender ratio (1339 F:595 M) and age of patients (54 ± 17 years, range 12–89 years) did not vary significantly during the study period. Indication for surgery was hyperthyroidism (n = 192), malignancy (n = 239), multinodular goitre (n = 833), primary hyperparathyroidism (n = 670). Main operating surgeon was a Consultant (n = 2) or an advanced surgical trainee (n = 3).

During each time period, LOS decreased from 2.8 ± 2.5 to 1.9 ± 2.5 days. The percentage of patients discharged on the day of their operation increased progressively from 20 to 45% for thyroid surgery and from 30 to 75% for parathyroid surgery. Readmission rate was 0.8%. There was no peri-operative mortality. Introduction of routine short-term hypocalcaemia prophylaxis (2000), scan-directed parathyroidectomy (2003), Harmonic scalpel (2005) and local anaesthetic parathyroidectomy (2009) had impacted on LOS during the study period. At the present time patients expect to be discharged on the day of surgery for parathyroid surgery and on postoperative day 1 after total thyroidectomy for benign disease. According to official DOH figures the current LOS for our unit is the shortest in the UK.

Conclusion  
Reducing LOS for thyroid and parathyroid surgery has been facilitated by pioneering acceptance of new technologies and by a ‘critical mass’ effect that allows a confident multidisciplinary approach with safe outcomes that can complement likely financial benefits (though not formally assessed in this study).

Thyroid swelling and tracheal compression: a cautionary tale  
Matthew Fenech, James MacKay, Simon Pain & Francesca Swords  
Norfolk and Norwich University Hospital, Norwich, UK.

A 28-year old Lithuanian woman presented with a 6-week history of firm anterior neck swelling. There were no symptoms of local obstruction, invasion, or thyroid dysfunction, and no past medical history, medication use or relevant family history. On initial assessment, she was euthyroid, with a 6 cm hard mass replacing the right lobe of the thyroid, a 2 cm lymph node lateral to it, but no other lymphadenopathy. No cellular material could be obtained on initial clinical and ultrasound-guided fine-needle aspirates. An ultrasound-guided core biopsy of the thyroid mass revealed a diffuse inflammatory cell infiltrate comprising lymphocytes and eosinophils, no thyroid follicles, and extrathyroidal fibrosis. These appearances were thought to be in keeping with Reidel’s thyroiditis.

The patient then deteriorated, developing stridor, dysphagia and snoring. Her tracheal diameter fell from 8 to 4 mm over a 6-week period. She was admitted immediately and started on 1 mg/kg oral prednisolone. Within hours of the first dose, her symptoms improved. Her FEV1 rose from 2.54 to 2.81 over 48 h, accompanied by a clinically appreciable 20% reduction in the size of the mass. Though the histology was compelling for Reidel’s thyroiditis, this was not in keeping with the clinical finding of lymphadenopathy. She was also perceived to be at increased risk of papillary thyroid cancer, due to childhood exposure to radiation from the Chernobyl disaster. Lymph node biopsy was therefore performed prior to starting prednisolone therapy. This revealed a mixed lymphoid population and Reed Sternberg cells staining for CD30 and CD15. Subsequent immunohistochemical staining of the thyroid biopsy revealed the same appearances, and the patient is now responding to ABVD chemotherapy.

Intrathyroidal Hodgkin’s lymphoma is a rare form of a rare disease – in 6 series comprising 248 cases of thyroid lymphoma, only 3 patients had Hodgkin’s lymphoma – and an unexpected underlying diagnosis in this case.

Ectopic prolactinoma in a patient with a clivoid mass  
Patrice Queenan & Tristan Richardson  
Royal Bournemouth Hospital, Bournemouth, UK.

We present a case of a 71-year-old gentleman who presented with a clivoid mass to the ophthalmologists.

The patient presented with left retro-orbital pain. He was generally fit and well, but his past medical history included cancer of the prostate and gout. An MRI brain was performed, which demonstrated a lesion between the left internal carotid artery and the clivus. CT chest/abdomen/pelvis confirmed no evidence of metastatic prostate cancer.

Interval scanning was performed at four months and demonstrated an increasing lesion size involving the clivus. The pituitary was of normal size, shape and position. The patient was referred to the Neurosurgeons. The differential diagnosis of a chordoma or clival tumour were considered. An excision biopsy was performed via image-guided endoscopy. There were no postoperative complications.

Histology was consistent with a prolactinoma. Prolactin levels were then measured and found to be elevated at 9500 μl (reference range <650 μl). The pituitary gland was not felt to have been disturbed during surgery.

The patient was referred to Endocrinology for follow up and dynamic testing. He was commenced on Cabergoline with a reduction in the serum prolactin to 1677 μl.

Tumours arising at the clivus are uncommon. However, the differential diagnoses to be considered are chordomas, meningoalomas, metastases, and pituitary macroadenomas. Investigation of these patients should always include measurement of serum prolactin in order to prevent unnecessary and potentially harmful surgical intervention.
Prevalence and prediction of endocrinopathies in thalassaemia major
Ploutarchos Tzoulis, Ai Leen Ang, Farrukh Shah & Maria Barnard
The Whittington Hospital NHS Trust, London, UK.

Aims
Endocrine deficiencies are common complications of thalassaemia major. Our haematology department serves one of the UK’s largest populations of thalassaemia patients. We assessed our thalassaemia major patients’ endocrine status and factors associated with endocrinopathies.

Methods
Retrospective analysis of our thalassaemia major patients on active follow-up in April 2010. Parameters such as age, gender, hepatitis C infection, compliance with chelation therapy, mean ferritin over 10 years, highest Ferrisican liver iron concentration from 2007 to 2010, lowest cardiac magnetic resonance T2* value between 1999 and 2010 were tested for independent association with each endocrinopathy, using multivariate analysis.

Results
Data was reviewed from 102 thalassaemia major adults (52 females, 50 males) with median age 35.5 years (range 17–58 years). Clinical hypothyroidism was recorded in 11.8% of patients (9.8% primary, 2.0% secondary), subclinical hypothyroidism in 2.9%, normal thyroid status in 85.3%. Prevalence of hypoparathyroidism was 13.7%. Hypothyroidism and hypoparathyroidism were not independently related with any of the parameters tested. Hypogonadal/hypogonadism was recorded in 64.7% and primary hypogonadism in 2.9%. Hypogonadism was associated with myocardial iron loading. For every 1 ms decrease in the lowest cardiac magnetic resonance T2* value between 1999 and 2010, there was a 4.4% (95% CI 0.8–7.8%, P value 0.016) increase in odds of having hypogonadism.

No cases of GH deficiency or glucocorticoid deficiency were documented.

Conclusions
A significant proportion of thalassaemia major patients have endocrine deficiencies. Ferritin concentrations are not a reliable predictor of endocrine deficiency. Patients with evidence of cardiac iron loading over the previous 11 years were more likely to have hypogonadism. This suggests that tissue iron loading is an important factor leading to hypogonadism. Controlling total body iron in thalassaemia major is critical in preventing damage to multiple major organs.

Significant hirsutism complicating pregnancy with postpartum resolution
Mohammed Adlan & Lakdasa Premawardhana
Caerphilly Miners’ Hospital, Caerphilly, UK.

Introduction
A 28-year-old primigravida developed increasing hairgrowth in androgen sensitive areas in the first trimester of her first pregnancy. She was previously well with menarche at 14 years, normal periods thereafter and no difficulty in conceiving. She took no medication. Clinically, at 34 weeks gestation she had significant hirsutism of her face, arms, legs and a prominent male escutcheon. She was obese but not Cushingoid.

Investigations and results

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>34/52 gestation</td>
</tr>
<tr>
<td>Testosterone</td>
</tr>
<tr>
<td>Androstenedione</td>
</tr>
<tr>
<td>DHEAS</td>
</tr>
<tr>
<td>SHBG</td>
</tr>
<tr>
<td>UFC</td>
</tr>
</tbody>
</table>

Urinary androstenedione metabolites, androsterone (4000) and androsterolone (3000) were significantly elevated compared to pregnant women of a similar gestational age (<1000 µg/g creatinine), and investigations for CAH were normal. MRI scans of the abdomen and pelvis revealed a normal female foetus, normal adrenal glands but her ovaries were not visible. A postpartum transvaginal ultrasound scan revealed polyovular cysts but no tumour. She delivered a healthy, normal female child and her hyperandrogenism (Table 1) and hirsutism resolved completely postpartum.

Discussion
Although pregnancy is a ‘pseudocushingoid’ state, symptomatic hyperandrogenism occurs only rarely. In hyperandrogenic states, the foetus is protected from virilization by several mechanisms. The cause for hirsutism in our patient was clearly reversible in the postpartum period and suggested a benign pregnancy specific source. Although a luteoma of pregnancy or hyperreactio luteinialis (both of which regress after delivery) were possible diagnoses, she was a Caucasian and these usually large tumours were not visible on MRI scanning. The appearances of the ovaries postpartum on ultrasound scanning indicates PCOS (unlikely) or the hyperandrogenism during pregnancy.

A case of cerebral salt wasting syndrome
Simmi Krishnan, Kalpana Katshai & Simon Howell
Royal Preston Hospital, Lancashire, UK.

A 55-year-old lady was admitted with sudden onset headache, diplopia, photophobia and confusion. CT brain showed SAH with hydrocephalus. Coiling of the aneurysm with EVD insertion was done on the same day. On day 5 postoperatively she developed hyponatraemia and dropped her GCS to 10/15. On examination she was volume depleted with dry mucous membranes and loss of skin turgor. Urine output over the previous 4 days had been 2.5–4 l/day, urinary sodium was elevated at 195 mmol/l with low serum sodium of 123 mmol/l. In view of the high urine output and clinical signs of hypervolaemia, cerebral salt wasting syndrome (CSWS) was felt to be the most likely diagnosis. She was treated with i.v. 0.9% saline (200 ml/h), and CVP monitoring was instituted. After 2 h, her serum Na had dropped to 117 mmol/l, and therefore 0.9% saline was changed to hypertonic saline (1.8%). By the following day GCS (14/15) and serum Na (129 mmol/l) had improved. However, on switching to 0.9% saline the serum Na dropped to 123 mmol/l and therefore hypertonic saline was recommenced. By day 9 serum Na had stabilised at 127 mmol/l and she was switched to normal saline.

The next day her serum Na dropped again to 119 mmol/l with persistently elevated urinary sodium (225 mmol/l) and high urine output. She was therefore changed back to hypertonic saline and this was continued until her urinary sodium level was <150 mmol/l.

By day 15, her serum Na was stable at 134 mmol/l, urinary sodium was 100 mmol/l, and GCS had improved to 15. Normal saline was recommenced with no drop in serum Na, and she was gradually weaned off i.v. fluids.

Treatment of CSWS requires replacement of excreted sodium, which can usually be achieved with i.v. normal saline. If sodium losses are very large then hypertonic saline may be required.

Milk–alkali syndrome: not to be forgotten
Ramona Vergaduer & James Lawrence
Salisbury Foundation Hospital NHS Trust, Salisbury, Wiltshire, UK.

A 50-year-old woman with learning difficulties and schizophrenia presented to accident and emergency with 5 days of epigastric pain and vomiting. Initially, she was peripherally shut down, with a pulse of 120 and blood pressure of 115/80. Chest examination was normal. Abdominal examination showed epigastric tenderness with guarding.

Blood tests confirmed a diagnosis of acute pancreatitis (Amylase 1972 IU/l), with hypercalcemia (corrected calcium 3.46 mmol/l) and acute renal failure (creatinine 692 mmol/l, urea 31.8 mmol/l).

Initial management included i.v. fluids, i.v. antibiotics and transfer to high dependency unit. The following day her bloods were as follows: Corrected Calcium 2.64 mmol/l, PTH 17.6 pmol/l.

On examination she was volume depleted with dry mucous membranes and loss of skin turgor. Urine output over the previous 4 days had been 2.5–4 l/day, urinary sodium was elevated at 195 mmol/l with low serum sodium of 123 mmol/l. In view of the high urine output and clinical signs of hypervolaemia, cerebral salt wasting syndrome (CSWS) was felt to be the most likely diagnosis. She was treated with i.v. 0.9% saline (200 ml/h), and CVP monitoring was instituted. After 2 h, her serum Na had dropped to 117 mmol/l, and therefore 0.9% saline was changed to hypertonic saline (1.8%). By the following day GCS (14/15) and serum Na (129 mmol/l) had improved. However, on switching to 0.9% saline the serum Na dropped to 123 mmol/l and therefore hypertonic saline was recommenced. By day 9 serum Na had stabilised at 127 mmol/l and she was switched to normal saline.

The next day her serum Na dropped again to 119 mmol/l with persistently elevated urinary sodium (225 mmol/l) and high urine output. She was therefore changed back to hypertonic saline and this was continued until her urinary sodium level was <150 mmol/l.

By day 15, her serum Na was stable at 134 mmol/l, urinary sodium was 100 mmol/l, and GCS had improved to 15. Normal saline was recommenced with no drop in serum Na, and she was gradually weaned off i.v. fluids.

Treatment of CSWS requires replacement of excreted sodium, which can usually be achieved with i.v. normal saline. If sodium losses are very large then hypertonic saline may be required.
Over the coming days she became more unwell and was intubated on intensive care unit. Her calcium plummeted and was managed with calcium infusion. She then slowly recovered and calcium normalised.

This audit was performed to assess management of thyroid carcinoma before the 15th day. She became confused and biochemically had SIADH. She was given Tolvaptan for 10 days. Hyponatraemia resolved rapidly and sodium increased to 133 mmol/l and remained stable. Patient 2, an 83-year-old woman with background problems including atrial fibrillation, was admitted electively for pacemaker insertion. Sodium on admission was 129 mmol/l. Following pacemaker insertion, she developed heart failure and needed diuretics, which were difficult to continue as she developed significant hyponatraemia. Her nadir sodium level was 114 mmol/l, leading to neurological sequelae. She was prescribed Tolvaptan for 8 days, which improved her sodium. Over the next few months her sodium levels have remained stable. Patient 3 was admitted with fractured neck of femur for 8 days, which improved her sodium. Over the next few months her sodium levels have remained stable. Patient 3 was admitted with fractured neck of femur and operated. Sodium on admission was normal, but dropped to 110 mmol/l by the 15th day. She became confused and biochemically had SIADH. She was initially managed with strict fluid restriction but this failed to improve matters. She was given Tolvaptan for 10 days. Hyponatraemia resolved rapidly and sodium levels remained normal since.

Discussion
Hospitalised patients can develop hyponatraemia due to a variety of causes and some can be very symptomatic. Tolvaptan use has been studied in multiple experimental and clinical trials which have used Tolvaptan for over 30 days. In clinical practice Tolvaptan appears to restore salt and water homeostasis rapidly following short-term use. Once the cause of hyponatraemia is treated normal sodium levels are maintained long-term.

Use of Tolvaptan in hyponatraemia: how long is treatment required?
Kalpita Majumdar & B S Aditya
Aintree University Hospitals NHS Foundation Trust, Liverpool, UK.

Introduction
Hyponatraemia is common biochemical abnormality in hospitalised patients and is difficult to manage in some cases. Tolvaptan is presently licensed in the UK for the treatment of hyponatraemia secondary to SIADH. We present our experience of using Tolvaptan.

Case
Patient 1 was admitted with sagittal sinus thrombosis. Over 9 days serum sodium dropped from normal to 121 mmol/l with biochemical evidence of SIADH. The patient was treated with Tolvaptan for 4 days, after management with fluid restriction and Demeclocycline had failed. Sodium increased to 133 mmol/l and remained stable. Patient 2, an 83-year-old woman with background problems including atrial fibrillation, was admitted electively for pacemaker insertion. Serum sodium on admission was 129 mmol/l. Following pacemaker insertion, she developed heart failure and needed diuretics, which were difficult to continue as she developed significant hyponatraemia. Her nadir sodium level was 114 mmol/l, leading to neurological sequelae. She was prescribed Tolvaptan for 8 days, which improved her sodium. Over the next few months her sodium levels have remained stable. Patient 3 was admitted with fractured neck of femur and operated. Sodium on admission was normal, but dropped to 110 mmol/l by the 15th day. She became confused and biochemically had SIADH. She was initially managed with strict fluid restriction but this failed to improve matters. She was given Tolvaptan for 10 days. Hyponatraemia resolved rapidly and sodium levels remained normal since.

References
1. Kalpita Majumdar & B S Aditya
Aintree University Hospitals NHS Foundation Trust, Liverpool, UK.

The thyroid and the skin
Niranjala Hewapathirana & Peter Mansell
Nottingham University Hospitals, Nottingham, UK.

Case
A 41-year-old presented to his GP with a pruritic skin rash affecting the whole body but sparing the face. He was treated with antihistamines with no improvement. On investigation thyroid function tests showed free $T_4$ 48 pmol/l, free $T_3$ 11.6 pmol/l with suppressed TSH <0.1 mU/l. He was started on carbimazole and referred to endocrinology. At the clinic it was noted that he has lost weight, felt hot all the time. There was no past history of rash. He was not on any medication prior to the rash. He had a wide spread urticarial skin rash, lid lag and a small diffuse goitre. The TPO antibodies titre was raised at 2857 IU/ml (0–60), normal ESR and normal vasculitc screen.

A skin rash as a presentation of thyrotoxicosis is a very rare finding. We sought a dermatology opinion but no alternative diagnosis was found. Our patient’s hyperthyroidism came under control with 30 mg of carbimazole once daily. Thyroid function tests improved and the rash lessened but was still troublesome. He was therefore referred for therapeutic radio-iodine as definitive treatment. The thyroid over-activity came under control requiring a small dose of carbimazole at present. The rash is much improved. Interestingly he had a flare up when he stopped carbimazole for two weeks around the time of radioiodine treatment, when there will have been a transient minor deterioration of thyroid over-activity.
Discussion
This case illustrates a rare presentation of thyrotoxicosis. The skin rash is thought
to be the result of thyroxine modulating the cAMP levels within the mast cells.
The condition is fairly resistant to symptomatic management with antihistamines
but generally regresses only once the hyperthyroidism is under control.

P83
An unusual case of medullary thyroid cancer presented with T3 toxicosis and type 2 diabetes
Pesh Amin
Royal Derby Hospital, Derby, UK.
A 76-year-old man with recent history of diet control diabetes and hypertension
presented to his GP with change in his voice and biochemical T3 toxicosis. He had
no further assessment and received no treatment. Few months later he presented
with right side goitre and found to have right vocal cord palsy and medullary
thyroid cancer and hyperthyroidism. He had evidence of local nodal and distant
metastases. Soon after biochemical euthyroidism he underwent total thyroid-
ectomy and local lymph node resection.
Thyroid cancer is occasionally associated with hyperthyroidism. Medullary
thyroid carcinoma (MTC) has been rarely described in association with Graves’
disease or other forms of hyperthyroidism. Careful evaluation of hyperthyroid
patients is always necessary to exclude the presence of associated malignancy and
to determine the most appropriate therapeutic plan.
A recent study presented at the International Thyroid Congress suggests that
endogenous subclinical types of hyperthyroidism may have a larger metabolic
effect on insulin resistance than the exogenous type of the condition. While it is
obvious that hyperthyroidism does not protect against thyroid cancer but in actual
fact it can have impact on insulin resistance and probably increasing chance of
mutation and thyroid cancer.
The MTC is not common and such presentation like our case is quite rare.
However, this case highlights the importance of clinical vigilance and proper
assessment of thyroid dysfunction. Further research studies are required to explore
cause and relationship between thyroid dysfunction, insulin resistance and
increase risk of thyroid mutation.

P84
Tramadol-induced adrenal insufficiency. A case report.
Sharon Chan, Miguel Debono & T Hugh Jones
Department of Endocrinology, Barnsley Hospital NHS Foundation Trust,
Barnsley, UK.
Background
The effect of long term opioids on the hypothalamo-pituitary–adrenal (HPA) axis
is conflicting. We present a case of a 21-year-old female who presented with
adrenal insufficiency (AI) secondary to chronic tramadol use.
Case summary
Our patient presented with a three year history of non-specific abdominal pain,
lethargy and dizziness. No cause was found for these symptoms despite thorough
investigations. One month before referral to Endocrinology outpatients, she was
hospitalised with a three day history of dizziness, vomiting and pressure-like
headaches. An MRI scan showed an incidental pituitary microadenoma whilst the
pituitary profile revealed a mildly abnormal Synacthen test with a baseline 0900 h
cortisol of 54 mmol/l and a 30-min cortisol of 537 mmol/l. Her medications included
Tramadol 50 mg TDS besides S budesonid, Metoclopramide, Omeprazole and
Ibuprofen. Her Synacthen test normalized when advised to stop tramadol.
Two months later, she was readmitted with similar symptoms. Tramadol 100 mg
QDS had been inadvertently restarted by her GP for persistent abdominal pain.
Cortisol levels from the Synacthen test were 45 and 307 mmol/l at 0 and 30 min
respectively. ACTH was relatively low at 9.7 ng/l. The rest of the pituitary profile
was normal. A diagnosis of tramadol-induced AI was made. Repeat Synacthen tests
and ACTH normalized when stopping tramadol. Her quality of life improved
significantly.
Conclusion
The sequence of events and development of AI on re-challenge with tramadol
support this drug as the cause for this event. To our knowledge this is the first
case of tramadol-induced AI although this effect on the HPA axis has been
previously reported with other opioids. There are currently no guidelines
recommending routine screening of adrenal status in patients on opioids but
clinicians should be aware of the possibility of AI in opioid users. Systematic
studies on the effects of opioids on the HPA axis are necessary.

P85
A case of carcinoid syndrome due to medullary thyroid carcinoma
Daniel Flanagan1,2, Thomas Fox1,2 & Jamie Fulton1,2
1Derriford Hospital, Plymouth, UK; 2Peninsula College of Medicine and
Dentistry, Devon, UK.
A 73-year-old man was referred to the general medical clinic with a 3-year history
of shortness of breath and wheeze. During assessment he commented that over
the same period he had also had intermittent sweats, flushing and redness of the face
especially after eating and taking red wine. Echocardiogram and urinary 24-h
5-hydroxyindoleacetic acid (5-HIAA) were arranged. Surprisingly two of three
urinary 24-h urinary 5-HIAA were positive (43 and 47.5 mmol/24 h and
30 mmol/24 h, NR 6–36 mmol/24 h).
Given the clinical suspicion of carcinoid syndrome an octreotide scan was
performed in anticipation of finding an intestinal lesion. Unusually this
demonstrated avid tracer uptake in the neck corresponding to a 3.3 cm mass
in the thyroid gland on computerised tomography. He was referred to the
endocrine services for further evaluation.
MIBG SPECT confirmed avid uptake in the right lobe of the thyroid gland.
Neither imaging modalities showed associated lymphadenopathy. The diagnosis
of a neuroendocrine thyroid tumour was made and ultrasound guided fine-needle
aspiration cytology of the thyroid mass was performed. Initial cytology indicated
a follicular lesion grade Thy 3. Repeat FNAC was more suggestive of a medullary
neoplasm. Core thyroid biopsy confirmed a medullary carcinoma of the thyroid
which was calcitonin positive. MRI of the adrenals and serum calcium were
normal making MEN2 unlikely.
The patient was commenced on lanreotide 60 mg monthly and underwent total
thyroidectomy and neck dissection. Histology showed a well-circumscribed
medullary thyroid carcinoma. He made an excellent post-operative recovery with
total resolution of his symptoms of flushing. Post-operative urine collections for
5-HIAA were normal, as were calcitonin and chromogranin A levels. A follow-up
octreotide scan 4 months after surgery showed no avid uptake in the neck.
Although carcinoid most commonly is derived from an intestinal tumour source it
is important to note that medullary thyroid carcinoma is also a rare source.

P86
Calcitonin negative medullary thyroid cancer
David Woods1,3, Anjali Santhakumar1, Sara Johnson1,3 & Sh Aspinall1,2
1Newcastle and Northumbria NHS Trusts, Newcastle, UK; 2University of
Newcastle, Newcastle, UK; 3Royal Victorian Infirmary, Newcastle, UK.
Medullary thyroid cancers (MTC) account for about 5% of thyroid cancers. The
biochemical hallmark of MTC is the secretion of calcitonin (CT). CT levels are
both a key feature of pre-operative diagnosis and post-operative follow up. CT
scoring in a cohort of over 10,000 patients with thyroid nodular disease has
demonstrated that a positive CT test has a higher diagnostic sensitivity and
specificity for MTC than fine needle aspiration (FNA). They may also secrete
carcinoembryonic antigen (CEA). We report a patient with histologically proven
MTC with negative CT and CEA pre-operatively who originally presented with
evidence of primary hyperparathyroidism.
Case presentation
A 63-year-old retired plasterer attended the endocrinology clinic with
biochemical evidence of primary hyperparathyroidism: calcium of 2.76 mmol/l
(2.12–2.60), parathyroid hormone (PTH) level of 76 ng/l (10–60), 24 h urinary
calcium of 11.2 mmol/24 h (2.5–7.5), vitamin D replete. Pre-operative
investigation revealed a 2.5% increase in 1.4 cm dominant left thyroid nodule which
was hypoechoic and associated with some calcification. FNA was reported as
THY3, consistent with a follicular neoplasm. Owing to the association of MTC
with primary hyperparathyroidism in MEN2 he had a pre-operative assessment of
CT and CEA. CT was reported on 2 separate occasions as <11 ng/l (<11) and
12 ng/l (<11). CEA was 4.1 ng/l (<5). Overnight urinary metanephrine and
normetanephrine levels as well as plasma metanephrines and normetanephrines
assessed pre-operatively were normal.
The patient underwent bilateral neck exploration with left hemithyroidectomy.
The overall histological features were consistent with medullary thyroid
carcinoma pT1b pNX pMX (TNM 7th edition). Immunohistochemistry showed
diffuse cytoplasmic positivity for CT and heterogeneous labelling with CEA. His
RET proto oncogene status is awaited. These patients may constitute a subset of
patients with “atypical” MTC where the tumour can produce CT (hence positive
staining) but is unable to secrete the protein.
A case of complete androgen insensitivity syndrome (CAIS), late presentation and difficult management
Shiva Mongolu, Jana Bujanova & Darryl Meeking
Queen Alexandra Hospital, Portsmouth, UK.

Introduction
Complete androgen insensitivity syndrome (CAIS) is an X-linked genetic disorder characterised by normal female appearance, including external genitalia and the presence of 46XY karyotype. We report a case of CAIS, diagnosed in adulthood, and discuss ethical issues surrounding the disclosure of diagnosis and associated difficulties in further management.

Case history
A 33-year-old Nigerian lady was referred to our endocrine service with Primary amenorrhoea. She stated that she was partially investigated in another centre and was found to have a shrunken uterus on ultrasound scanning and Laparoscopy, and normal ovaries. She had normal external genitalia with paucity of axillary and pubic hair. Although her testosterone level was markedly elevated at 21 nmol/l, she was not hirsute with no masculine features. Her DHEAS was normal (3.2 μmol/l) and 17OHP Progesterone was mildly raised (5 μmol/l).

Chromosomal analysis demonstrated 46XY karyotype consistent with androgen insensitivity syndrome. Each time the issue of chromosomal abnormality was mentioned, she declined further information and refused to see a clinical geneticist. Despite this, she was disclosed to the patient and her family but, she refused to accept stating her previous ‘normal’ investigations.

After constant persuasion, she underwent an MRI scan of her pelvis which confirmed absence of uterine tissue and presence of reproductive tissue within pelvis (probably testicular in origin). She refused referral to surgeons for surgical removal of the tissue, despite being warned of malignancy risk and did not attend her further follow-up appointments.

Discussion
This case highlights the psychological trauma which can be associated when faced with a diagnosis of CAIS and how they frequently avoid medical care. Sensitivity, awareness and a multi-disciplinary approach are needed in the management of these patients.

P89
Congenital adrenal hyperplasia: spontaneous resolution or cure?
Aysha Javed, Sherine Thomas, David Ewins, Niru Goenka & Frank Joseph
Countess of Chester Hospital, Liverpool Road, CH1 1UL Chester, UK.

We present the case of a woman who first presented at age 18 with hirsutism. Menarche had been normal and she had regular menses. She gave no past medical history except that at age 6 she had been admitted to hospital with tonsillitis that was complicated by diarrhoea and vomiting, drowsiness and hypotension. At that time, Na+ was 130 mmol/l and K+ 3.8 mmol/l. She was treated with antibiotics and fluids and improved; electrolytes returned to normal.

On examination her blood pressure was 140/105 mmHg. She had acne but no features of virilisation; her BMI was 20.8 kg/m².

Routine biochemistry was normal. Two random measurements of 17 hydroxy-progesterone (17-OHP) were raised at 47 and 55 mmol/l (1.4–12). Synaechton stimulation resulted in an increase of 17-OHP from 72 to 79 mmol/l and then 88 mmol/l at 30 and 60 min respectively. Peak cortisol response was suboptimal at 412 mmol/l. DHEAS was high at >26 μmol/l (1.8–10.2) with a high testosterone 5.1 μmol/l (1.4–3.8). A 24 h urine collection showed normal cortisol excretion at 498 nmol/24 h and cortisol suppressed after overnight dexamethasone of 1 mg.

A diagnosis of non-classical heterozygous congenital adrenal hyperplasia was made and she was started on prednisolone 2.5 mg in the morning and 5 mg at night.

Hypercalcaemia improved and due to over-suppression of 17-OHP, prednisolone was tapered and eventually stopped 9 years later at age 27. A repeat short synacthen test (SST) showed a 17-OHP peak of 20.0 mmol/l and a cortisol peak of 500 mmol/l, again suboptimal. She also tested negative for known 21-hydroxylase mutations and repeat androgen levels had normalised.

Repeated SSTs have resulted in suboptimal cortisol response but normal 17OHP responses but with no clinical features of androgenisation or hypoadrenalism. We have no explanation for the resolved abnormalities but she remains well having declined long term steroid therapy and has steroids at times of illness.
In Central and Eastern Cheshire, Secondary Care endocrinology/diabetes clinicians can now access a summary of the patient’s primary care electronic patient record (EPR), using a secure Web browser held by the GP provider EMIS. This improves decision making at the point of care. The clinicians, including consultants, SpRs, dieticians and specialist nurses can access details of medication, allergies, and previous diagnoses, available for 95% of attendees. The GP summary record appears on the screen as a ‘Read-only’ document. Consent is given by the patient and audited. Importantly, none of the patients participating in the project has refused access. Feedback has been extremely positive. They view sharing information as a way to improve their own healthcare and also health outcomes for the wider community. One patient said: ‘this is a big step forward for patient safety’. Another patient observed ‘why aren’t all services sharing my information like this’. Hospital clinicians can record certain details from the consultation into a template and write short notes to the GP, that are added to the patient’s record in real-time. The patient’s GP is then able to view this back at the practice and include the comments in their own record if they wish. This has the potential to feed directly into individualised care pathways. In a separate development, the anonymised search facility means that we can take a longitudinal view of trends, such as results of testing for thyroid hormone imbalance or testosterone deficiency in patients with diabetes across one or several Primary Care Trusts. This affords an audit tool that can assess real outcomes.

Conclusion
Access to primary care information affords a radically different perspective, enhancing the clinic consultation and facilitating cross-boundary working with patient satisfaction. It offers a potential frameshift in the management of long-term metabolic and endocrine conditions.

Primary care records in hospital clinics: implementing two-way communication in Cheshire, UK
Ram Prakash Narayanan1, Dhanya Kalathil2, Farheen Raza2, Elizabeth Jarman1, David Lowes1, M Zubair Qureshi1 & Adrian H Heald2
1Vascular Research Group, The University of Manchester, Manchester, UK; 2Leighton Hospital, Crewe, UK; 3Medical School, The University of Manchester, Manchester, UK.

Psychiatric illness: a cause and hurdle to management of nephrogenic diabetes insipidus
Lisa Turner, Giridhar Tarigopula, Olympia Koulouri & Marie-France Kong
Department of Diabetes and Endocrinology, University Hospitals of Leicester NHS Trust, Leicester, UK.

A 54-year-old lady with a 27-year history of schizoaffective disorder presented with shaking episodes, polyuria and polydipsia. She was found to have a serum sodium of 157 mmol/l. Of note, she had been on lithium for several years but this had stopped three months previously as her serum sodium was raised at 156 mmol/l. On admission her lithium level was undetectable, confirming no recent use. Serum osmolality was 343 mOsm/kg and urine osmolality 82 mOsm/kg, suggesting diabetes insipidus. Thyroid function tests and other pituitary function tests were normal. Initially desmopressin was given as a diagnostic test but this had no impact and her serum sodium rose to 167 mmol/l. Her urine osmolality did not rise above 159 mOsm/kg. A paired serum osmolality was 360 mOsm/kg. During admission her serum sodium peaked at 175 mmol/l. As a result of her mental health issues, she was not drinking adequately to compensate for the fluid loss and required i.v. supplementation of up to 7 l/day. Indomethacin 50 mg BD and subsequently Chlorothalidone 100 mg BD were started while encouraging her to increase her fluid intake. I.v. fluids were gradually weaned off. At discharge her serum sodium was 140 mmol/l, serum osmolality 302 mOsm/kg and urine osmolality 226 mOsm/kg.

Unfortunately she was readmitted one month later with dehydration and hypokalaemia and she remains an inpatient 4 weeks later. Her diabetes insipidus persists despite not having taken lithium for almost 6 months and it is compounded by her inability to maintain her fluid intake. Diabetes insipidus may persist for several months or years after discontinuing lithium. Management of our patient remains a challenge. Drugs have had little impact on her condition which is exacerbated by her underlying mental health problems.

Thyroid function monitoring in amiodarone treated patients: the need to increase awareness among prescribing physicians
Graham Dunthorne & Zayd Merza
Barnsley Hospital NHS Foundation Trust, Barnsley, UK.

Introduction
Amiodarone is a commonly prescribed antiarrythmic drug. It can cause potentially serious adverse effects involving the thyroid gland, lungs, liver and eyes. It is recommended that patients on amiodarone have regular monitoring tests.

Methods
We conducted an audit of patients who were prescribed amiodarone by various physicians in our hospital between January 2007 and March 2008. The data was obtained from the patients case notes. We looked at tests done at baseline and regular intervals thereafter up to two years, including thyroid, liver, renal and pulmonary function tests and chest X-rays.

Results
The case notes of 79 patients (52 males and 27 females) were reviewed. The mean age was 70 ± 11 years. The commonest indication for amiodarone was atrial fibrillation (73%), 55, 39, 32, 27 and 26 patients continued therapy at 3, 6, 12, 18 and 24 months respectively. Thyroid function tests were performed in 78% of patients at baseline, 31% at 3 months, 64% at 6 months, 53% at 12 months, 55% at 18 months and 35% at 24 months. Liver function tests were performed in 82% of patients at baseline, 59% at 6 months, 50% at 12 months, 48% at 18 months and 31% at 24 months. Urea and electrolytes were checked in 95% of patients at baseline. Chest radiography was performed in 48% of patients at baseline, 9% at 12 months and 4% at 24 months. Pulmonary function tests were performed in 9% of patients at baseline.

Conclusion
This audit shows that a significant number of patients on amiodarone do not get the recommended regular monitoring for potential adverse effects of amiodarone. This highlights the need to increase awareness among physicians so that abnormalities can be detected at an early stage.

Pseudo-Cushing’s syndrome and central pontine myelinolysis
Marie-France Kong1, Kaustubh Nisal1, Michael Knopp1, Siew Lee Wong2, Yusuf Rajabally2 & Trevor Howlett1
1Department of Diabetes and Endocrinology, University Hospitals of Leicester NHS Trust, Leicester, UK; 2Department of Neurology, University Hospitals of Leicester NHS Trust, Leicester, UK.

A 52-year-old man was referred in June 2009 with weakness, unsteadiness, diarrohoea and weight loss of 14 kg in the last 4 months. His past medical history included hypertension, ischaemic heart disease and chronic obstructive pulmonary disease. He was a heavy smoker and admitted to drinking heavily for the past 9 months. His serum sodium was 126 mmol/l, serum potassium 2.0 mmol/l, serum bicarbonate 31 mmol/l. His chest X-ray was normal. He had persistent hypokalaemia for 4 months which had been attributed to his diarrohoea. His serum potassium was normal in December 2008. A 24 h urinary free cortisol was very high at 1491 (normal range 28–221 nmol/24h). However, he had no stigmata of Cushing’s disease. In view of his weight loss, smoking history and hypokalaemia ectopic ACTH syndrome was considered. He did not suppress fully after a 48 h high dose dexamethasone suppression test. ACTH was 6 ng/l. A CT thorax/abdomen/pelvis showed no evidence of a neoplasm and no adrenal mass. MRI of the pituitary gland was unhelpful as he was unable to keep still. A 24 h urine collection for 5-HIAA to exclude carcinoid syndrome was normal. Corticotrophin-releasing hormone stimulation test did not support a pituitary source of ACTH. He was readmitted a month later with recurrent hypokalaemia and muscle weakness. Serum sodium was 114 mmol/l, serum potassium 2.2 mmol/l. Over the next few days he became confused and agitated. A CT head scan excluded a subdural haematoma. CSF culture was negative.
An EEG showed mild diffuse slowing. MRI of the brain showed features compatible with central pontine myelinolysis. We concluded that our patient’s cortisol abnormalities was due to pseudo-Cushing’s syndrome caused by his excessive alcohol consumption which also accounted for his hypokalaemia and hypoglycaemia. Some patients have no physical evidence of glucocorticoid excess like in our patient.

P95

Cyclical Cushing’s or poorly controlled diabetes in an insulin resistant patient?
Marie-France Kong, Giridhar Tarigopula, Olympia Koulouri, Lisa Turner & Robert Gregory
Department of Diabetes and Endocrinology, University Hospitals of Leicester NHS Trust, Leicester, UK.

A 30-year-old Caucasian lady was diagnosed with type 2 diabetes in July 2005 on an oral glucose tolerance test and was treated with metformin. Sixteen months later she presented with urinary symptoms and was noted to have 2+ ketonuria. Her blood glucose was 13.4 mmol/l. Her BMI was 26 kg/m² and she was noted to have Cushingoid habitus and anacanthosis nigricans. Her blood pressure was normal. Her serum sodium was 130 mmol/l, potassium 3.7 mmol/l and bicarbonate 8 mmol/l. She was noted to have severe hypertriglyceridaemia of 58.4 mmol/l. Serum cholesterol was 16.1 mmol/l. She was treated with intravenous insulin and discharged home on twice daily pre-mix insulin. Investigations for Cushing’s syndrome were negative. 24 h urinary free cortisol was normal. 0900 h serum cortisol after overnight dexaamethasone suppression test was 36 mmol/l. ACTH was 6 ng/l. Cyclical Cushing’s was considered but six 24 h urinary free cortisol collections were negative. Despite taking ~300 units of insulin her HbA1c remained above 11% on a basal bolus regimen. Anti GADA was negative. Genetic analysis confirmed severe hyperinsulinaemia suppression with dyslipidaemia with very low adiponectin and high leptin levels, in keeping with patients with Cushingoid body habitus. On the basis that she could have a PPARY gene mutation she was commenced on pioglitazone with marked improvement of her diabetes control and sharp reduction in insulin requirement. HbA1c improved from 11.8 to 7.5% in 3 months. Subsequently the sequence of her PPARY gene has been reported to be normal. Her diabetes control has subsequently deteriorated with her most recent HbA1c being around 12%. Should we still investigate further for cyclical Cushing’s syndrome?

P96

Recreational jaundice
Rajesh Gupta, Damodharan Suresh & Vrijraj Rathod
Mid Essex Hospital NHS Trust, Chelmsford, Essex, UK.

A young fit male readmitted with three weeks history of malaise, pale stool, dark urine, pruritus with recent travel to Greece. He denied alcohol, illicit drug abuse. Examination revealed jaundice.

Investigation showed cholestatic liver impairment with Bilirubin: 448 μmol/l (7-35), ALT: 134 IU/l (17–63), ALP: 190 IU/l (32–91), HDL 0.34 mmol/l (0.9). Viral Screen, autoantibody, porphyria and tumour markers were negative. CT Abdomen showed tiny gall stone with normal bile duct and pancreas. MRI MRCP was normal.

Testosterone: 11.7 nmol/l (0.27), 17 B Oestradiol: 556 pmol/l (up to 73), LH: 4 U/l (0.7–11), FSH: 1 U/l (0.8–7.7), TFF: Normal, SHBG: 30.3 nmol/l (13–71), PRL: 165 mU/l (0–280).

Cause of Cholestasis remains unidentified at this point. Pt admits taking Creatin/Protein powder but denied taking any steroid. When asked repeatedly informed taking ALPHA SD (2a 17a dimethyl eticholan 3-one 17b-01) 10 mg BD for one month. Patient improved without treatment after stopping AAS.

Impression
Anabolic steroid induced cholestasis and deranged lipid profile.

Discussion
Anabolic-androgenic steroids (AAS) are the synthetic derivatives of testosterone and altered to reduce metabolism, achieve desirable anabolic effects and difficult detection. AAS use is associated with side effects of Cardiovascular, hepatic, gynaecological, behavioural, skin and endocrinological disorders.

Hepatic
With AAS abuse there is an elevated risk for liver tumours, cholestasis, toxic hepatitis and peliosis hepatitis. This is likely due to the liver being the primary site of steroid clearance. The alkylated AAS are highly hepatotoxic.

Cardiovascular
AAS can induce hypertension, MI, abnormal lipoproteins and possibly LVH. AAS can affect lipid profile adversely leading to reduction in HDL cholesterol raised LDL leading to early atherosclerosis.

Conclusion
As there is rise in unregulated abuse of AAS, so we need to be aware and consider anabolic steroid as cause of unexplained liver failure, deranged lipid profile and endocrinological abnormalities.

Main treatment is stopping the drugs and monitoring regularly.

P97

Hypercalcaemia following parathyroidectomy in a pregnant lady with MEN-1
Rhodri King, Emma Ward, Andy Scarsbrook & Steve Orme
Leeds Teaching Hospitals NHS Trust, Leeds, UK.

We present a 20-year-old lady who was known to have MEN-1 and had previously been treated for hyperparathyroidism at a different hospital in 2003 with excision of right upper and lower and left lower parathyroid glands and left thyroid lobectomy, resulting in normalisation of adjusted calcium (adjCa) levels. She presented to our department with persistently elevated adjusted calcium levels (adjCa 2.69 mmol/l) along with raised parathyroid hormone (PTH 16 pmol/l) and low total vitamin D levels (14.2 nmol/l). She was also roughly 8 weeks pregnant at this stage. Correction of her vitamin D deficiency did not improve her biochemistry and in July 2010 she was now 15 weeks pregnant with adjCa 2.86 mmol/l, PTH 10.6 pmol/l and total vitamin D 139 nmol/l. An ultrasound of her neck demonstrated a normal right thyroid lobe and no masses and a foetal anatomy scan was normal other than a possibility of a hyper echoic bowel.

After discussion with the radiology department she had an MRI of her neck and thorax which demonstrated a solitary parathyroid adenoma in the lower pole of the right lobe of the thyroid, likely to be an ectopic PTH adenoma. A focussed neck ultrasound confirmed a uniformly echo poor lesion measuring 16 mm × 7 mm with blood flow within it. Fluid aspirated from the nodule under ultrasound guidance had elevated PTH levels (280 pmol/l) consistent with an ectopic PTH adenoma. The lesion was excised during her second trimester and post-operatively her biochemistry normalised (adjCa 2.39 mmol/l, PTH <0.3 pmol/l).

This case illustrates the need for long term monitoring and follow up of MEN patients. It was complicated further by pregnancy, and careful choice of imaging enabled prompt diagnosis and treatment to minimise foetal complications of hypercalcaemia.

P98

Disappearing adrenal insufficiency
Earn H Gan1, Andy James2 & Simon H S Pearce1,2
1Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, UK; 2Endocrine Unit, Royal Victoria Infirmary, Newcastle upon Tyne, UK.

After adrenal insufficiency is confirmed by synacthen testing, lifelong steroid replacement is expected. We report a case of apparent reversal of adrenal insufficiency. Case details: a 66-year old man was referred for oral pigmentation, and a random cortisol level of 184 nmol/l. He reported a 2-year history of tiredness, nocturia, dry mouth and reduced libido. There was no dizziness,
salt-covorng, or weight loss. He denided taking any form of steroid or over-the-
counter drugs. Examination showed punctate oral pigmentation and a pigmented
patch on his finger, without generalised pigmentation. His blood pressure was
118/72 mmHg with a postural drop of 10 mmHg. He had a raised HbA1c (7.6%) and
mild hyponatraemia (134 mmol/l). A 250 μg synacthen test showed a basal
cortisol of 288 nmol/l; 30 min at 182 nmol/l and 60 min at 143 nmol/l. Hydrocortisone
(20 + 10 mg daily) was instigated with the diagnosis of probable Addison’s disease. However, his ACTH level came back at 25 ng/l (5–55 ng/l);
plasma renin and aldosterone levels were normal. Basal measurements of other
anterior pituitary hormones and paired plasma/urine osmolality were normal. Adrenal
autoantibody test was negative. He was well taking hydrocortisone for 6 months but his HbA1c worsened to 10%. The original diagnosis of adrenal failure was re-considered. Serum DHEAS levels
were normal, and there were detectable cortisol and aldosterone precursors in the
urine. The hydrocortisone dose was weaned and repeat short synacthen testing
demonstrated a basal cortisol of 258 nmol/l; peak 672 nmol/l. The diagnosis of
adrenal insufficiency was revoked. This case illustrates the need to make a clear
aetiological diagnosis of adrenal insufficiency. The abnormal initial synacthen
test result could have been due to either unwitting exogenous steroid
administration, transient isolated ACTH deficiency or failure to administer the
ACTH1–34 during the synacthen test. The normal serum DHEAS and the urine
steroid profile were helpful clues to the safety of a staged withdrawal of
hydrocortisone.

P99

Metastatic insulinoma in a patient with type 2 diabetes mellitus: case report
Noor Muhammad Abbassakoor\textsuperscript{1}, Marie-Louise Healy\textsuperscript{1}, Donal O’Shea\textsuperscript{2},
Donal Maguire\textsuperscript{2}, Cian Muldoon\textsuperscript{1}, Kieran Sheahan\textsuperscript{2} & Dermot O’Toole\textsuperscript{1}
\textsuperscript{1}St James’ Hospital, Dublin, Ireland; \textsuperscript{2}St Vincent’s University Hospital,
Dublin, Ireland.

Introduction
Insulinoma is a tumour, derived from the beta cells of the pancreas. The incidence
in the general population is 4 cases per million a year. 80 to 90% of insulinomas
are benign and <10% are malignant.

Case presentation
A 67-year-old lady was admitted via the emergency department after being found
unresponsive at home. She was found to be hypoglycaemic and responded to i.v.
dextrose. She was diagnosed with type 2 diabetes mellitus a year ago and had been
suffering from episodes of confusion and had significant weight loss over 1 year.
A 72 h fast test was stopped after two hours as she suffered another episode of
hypoglycaemia. Laboratory investigations revealed low blood glucose and
elevated C Peptide levels and elevated insulin levels. She underwent CT
Abdomen and Endoscopic Ultrasound which revealed a 6 cm pancreatic
hypoechoic lesion and large volume right sided liver metastases. Ultrasound
guided biopsy confirmed a pancreatic neuroendocrine tumour. The patient
underwent successful one-step RO surgical resection – distal pancreatectomy,
splenectomy and right hepatectomy and was recurrence free 12 months post
operatively. Her only residual problem is diabetes mellitus.

Conclusion
This case highlights a rare cause of hypoglycaemia in a diabetic patient. Medical
treatment with diazoxide and somatostatin analogues can be used until
oncological resection. Surgical resection of insulinomas remain the main curative
treatment where possible.

P100

The beneficial effects of long-acting octreotide in a patient with concomitant metastatic
neuroendocrine tumour and anaemia due to lower digestive bleeding
Saket Gupta, Lisa McGowan, Donal O’Shea & Gianluca Tamagno
Department of Endocrinology and Diabetes Mellitus, St Vincent University
Hospital, Dublin, Co. Dublin, Ireland.

Somatostatin analogues (SA) represent the most effective medical treatment for
the control of neuroendocrine tumour (NET) symptoms, like carcinoid syndrome.
Recently there has been evidence of lengthened time to tumour progression in
patients with midgut carcinoid treated with octreotide. Nevertheless, a number of
new therapeutic indications and the clinical effectiveness of SA for other clinical
conditions are appearing. A 69-year-old man was investigated for anaemia
(haemoglobin: 8.0 g/dl). He required blood transfusion every 2–3 weeks. He had a
medical history of peripheral vascular disease, coronary artery disease and
by-pass graft, decreased left ventricular function, and pulmonary hypertension.
Following a set of investigations, chronic digestive bleeding likely secondary to
angiodysplasia was diagnosed but surgical approach was not feasible. The
radiological investigations incidentally discovered abdominal and inguinal lymph
node metastases and subsequent lymph node biopsy revealed metastatic NET.
Unfortunately, the primary tumour remained unidentified. Treatment with long-
acting octreotide was established. The need for blood transfusions dramatically
decreased starting from the first month of treatment with long-acting octreotide.
His haemoglobin levels improved and remained stable around 10.0 g/dl over the
following 12 months. Since commencement of octreotide, he required one blood
transfusion every 4–5 months only. No adverse events occurred and the patient
remained stable from a NET-related point of view. To the best of our knowledge,
this is the first report of a serendipitous effect on anaemia due to chronic lower
digestive bleeding achieved by long-acting octreotide treatment prescribed for
metastatic NET. This report is consistent with the literature reporting a potential
increase in activity of SA for the control of NET symptoms, and even improvement
of evoked bleeding from a known or unknown source. In our opinion, this report shows that treatment with SA in patients with lower digestive bleeding can be beneficial if the surgical approach is not feasible and only palliative therapies are available.
lymphadenopathy and systemic examination was otherwise normal. His initial investigations showed hyponatraemia with serum sodium 121 mmol/l, potassium 3.4 mmol/l, serum osmolality 255 mosm/kg, and urine osmolality 672 mosm/kg. The rest of blood tests including: thyroid function, synchretin test, full blood count, liver functions, urea and creatinine were all normal. Chest X-ray showed consolidation at the left base. He was treated for pneumonia with secondary SIADH.

He returned 1 month later with anorexia, weight loss, night sweats and unsteadiness, with a sodium of 122 mmol/l. He underwent CT thorax and abdomen which revealed 5 mm pulmonary nodule. His case was discussed in the chest multidisciplinary team meeting with a decision to monitor this nodule with interval CT scanning to exclude an underlying neoplasm or TB. Tumour markers were normal and sodium improved with demeclocycline.

Several months later – repeat CT showed the pulmonary nodule had increased to 6 mm with hilar and abdominal lymphadenopathy. He was referred to the tertiary centre where the nodule was excised and found to be benign.

He was admitted later with fever, night sweats and weight loss. He had oral candidiasis. An HIV test was carried out and reported positive. Subsequently, he was found to have pneumocystic jiroveci, syphilis, cytomegalovirus (serologically), B-cell lymphoma, HIV encephalopathy and he was transferred to the infectious diseases unit for treatment.

Hyponatraemia can be associated with HIV for multiple reasons including SIADH (secondary to pulmonary and CNS infections or malignancy), adrenal insufficiency (usually infective involvement of the adrenal glands), HIV enteropathy, HIV-associated nephropathy or direct infection of the posterior pituitary (CMV).

Learning points
1. Hyponatraemia can be associated with HIV and AIDS.
2. HIV should be part of the differential diagnosis in unexplained hyponatraemia, even in ‘low risk’ patients.

### P104

**Unwanted recovery from secondary hypogonadism: paradoxical effect of Zoladex**

Suresha Muniyappa, Ruth MacInerney & Rob Robinson

Chesterfield Royal Hospital, Chesterfield, UK.

We present the case of an 80-year-old man found to have a non-functioning pituitary macroadenoma causing secondary adrenal insufficiency and hypogonadism in 2006. Thyroid function and prolactin levels were normal. With hydrocortisone replacement he was doing well. There was no visual field defect and he continued with conservative management. On enquiry about testosterone treatment, it had been started in Oct 2008, but discontinued within a month as there was no change in his wellbeing. A DEXA scan was normal. His testosterone levels remained low, between 4.3 and 5.6 mmol/l. PSA was 4.3 μg/l and rectal examination was normal.

Testosterone therapy was discussed and re-started in May 2009. His PSA, however, rose to 12.2 μg/l in July 2009. Testosterone replacement was stopped immediately, with the PSA level falling to 4.3 mmol/l. A biopsy of his prostate confirmed adenocarcinoma of prostate and he was referred to the oncologists. LHRH agonist therapy (Zoladex) was started with cyproterone acetate cover, aiming for chemical castration, in December 2009. After one dose, testosterone levels in February 2010 had surprisingly risen to 15.8, with PSA of 12.2 μg/l. With this paradoxical result and after endocrine consultation, he had bilateral orchidectomy to achieve a testosterone level consistent with castration. Post operatively, testosterone fell down to 0.4 mmol/l with PSA of 0.4 μg/l. He also had radiotherapy to the prostate. Tumour reduced in size from T1 to T0. He remains under regular follow up.

This case highlights a prolonged ‘flare’ effect after LHRH agonist therapy in a man with a non-secretary pituitary adenoma. Normally after such therapy there is a surge in LH lasting about 1 week, before levels fall. In this case testosterone levels were elevated more than two months after such therapy, suggesting a paradoxical response of his pituitary gonadotrophs to LHRH agonist therapy.
Endocrine Abstracts

GLP-1 stimulates β-cell proliferation, it also enhances the differentiation of new beta cells from progenitor cells in the pancreatic duct epithelium and it is capable of inhibiting apoptosis of β cells. GLP-1 also exhibits other effects of importance for glucose homeostasis, such as, inhibiting glucagon secretion, delaying gastric emptying, and stimulating insulin biosynthesis. These effects come along with a potential increase in peripheral insulin action. Type 2 diabetic patients have an impaired secretion of GLP-1. On the other hand, patients with type 2 diabetes mellitus are considered to be at high risk for cardiovascular disease. A mutual relationship exists between insulin resistance and heart failure. High affinity receptors for GLP-1 are present in the heart and vascular tissue, so it is conceivable that the incretins may improve cardiovascular function.

Aim

The aim of this study was To assess the plasma level of GLP-1 in patients with congestive heart failure, both those with type 2 diabetes mellitus and those who are non diabetic and to assess the possible role of GLP-1 in the development of congestive heart failure.

Method

Our study included 54 subjects, divided into 4 groups; ‘group 1’ included 15 non diabetic patients with congestive heart failure, ‘group 2’ included 15 type 2 diabetic patients with congestive heart failure, ‘group 3’ included 12 type 2 diabetic patients with normal cardiac function and ‘group 4’ included 12 normal control subjects. All subjects were submitted to full clinical and biochemical assessment, Fasting plasma glucagon-like peptide 1 measured by ELISA and echocardiogram.

Results

In the presenting study fasting serum GLP-1 was found to be significantly lower in cases suffering of either heart failure or type 2 DM in comparison to those healthy control cases. Furthermore, diabetic cases with heart failure showed significant lower level of serum GLP-1 in comparison to non-diabetic heart failure cases. Therefore, our results may suggest a role for GLP-1 deficiency in development of congestive heart failure either in type 2 diabetic or non-diabetic cases.

Cytokines, growth factors, neuroendocrinology and behaviour

Altered corticosterone homeostasis in hippocampus leads to memory impairment in hypobaric hypoxia

Iswar Baitharu1, Salya Narayan Deep1, Vishal Jain1, Kalpana Barhwal2, Sunil Kumar Hota3, Dipit Prasad1 & Govindasamy Ilayvazghan3
1Defence Institute of Physiology and Allied Sciences, DRDO, Delhi, Delhi, India; 2Defence Institute of High Altitude research, DRDO, Leh, Jammu Kashmir, India.

Hypobaric hypoxia, an environmental condition arising due to the reduced partial pressure of oxygen on ascent to high altitude, is known to cause memory
impairment. The mechanism underlying the cognitive dysfunction has been attributed to oxidative stress, glutamate excitotoxicity and Ca²⁺ mediated death cascade. Though the role of corticosterone in higher order brain function including cognition has been well documented in restrained stress, social stress and other moderate stress, its impact in hypobaric hypoxia induced cognitive dysfunction still remains to be investigated. To determine the effect, five groups of rat were exposed in a simulated decompression chamber at an altitude of 25,000 ft for 0, 3, 7, 14 and 21 days. Corticosterone synthesis was blocked using metyrapone (75 mg/kg BW) on the day showing maximum corticosterone level and neuronal damage. Our study revealed a duration dependent elevation in corticosterone level in plasma and hippocampus. There was increased ROS generation, decreased antioxidant defence system and compromised neuronal energy status. Exposure to hypobaric hypoxia upregulated the expression of glucocorticoid and mineralocorticoid receptor in a duration dependent manner. Neuronal glucosel transporter Glu1 and blood–brain barrier glucose transporter Glut1 was found to be upregulated during initial period of exposure to hypobaric hypoxia. There was decreased citrate synthase activity and increased glutamate dehydrogenase activity along with altered expression of proteins (MDR1/ABG and HSD1) regulating the corticosterone bioavailability in hippocampus. These findings suggest that chronic elevation in corticosterone level may lead to induction of oxidative stress and neuronal dysfunction in higher order brain function.

P110
Selected adipocytokines serum levels in patients with ischemic stroke
Bogdan Marek, Radoslaw Bienek, Dariusz Kajdaniuk, Mariusz Nowak, Lucyna Sieminska, Joanna Glogowska-Szelag & Beata Kos-Kudla
Department Pathophysiology and Endocrinology, Silesian Medical University, Zabrze, Poland.

Adiponectin (ADPN), resistin (RSN) and leptin are proteins that affect insulin resistance and atherosclerosis significantly. Although low levels of adiponectin and high levels of resistin and leptin are associated with coronary heart disease and cardiovascular disease risk factors, it is unclear whether adiponectin, resistin and leptin levels are related to the risk of developing ischemic stroke. The aim of the study was to evaluate the adiponectin, resistin and leptin serum levels in patients with acute ischemic stroke and their role in the pathogenesis of cerebrovascular diseases. We examined 70 patients with acute ischemic stroke and 30 stroke-free subjects of similar age and sex distribution. In all subjects we examined lipid pattern (cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides), blood glucose level, blood pressure, BMI and HOMA-IR.

Results
In comparison with referents, patients with stroke had significantly higher leptin (14.98 vs 10.47 ng/ml) and resistin (28.92 vs 12.25 ng/ml) levels, but adiponectin (15.49 vs 14.32 ng/ml) did not differ between groups.

Conclusions
Despite moderate associations between adiponectin and cardiovascular disease risk factors, we found no evidence of an association between serum adiponectin levels and incident ischemic stroke. Leptin and resistin may are important links to the development of cerebrovascular disease.

P111
Abstract withdrawn.

P112
Characterisation of the stress–response in BACE1 heterozygous mice
Paul Meakin, Johnathan Winter, Mike Ashford & Alison McNeilly
University of Dundee, Dundee, UK.

Alzheimer’s disease (AD) is one of the most common causes of dementia world wide. Accumulation of β-amyloid (Aβ) has been implicated as a causative factor of AD. Beta secretase (BACE1) catalyses the rate limiting step in the production of Aβ and has been postulated as a potential therapeutic target for AD treatment. Environmental factors, including psychological stress, accelerate the development of dementia and AD. This study examined hypothalamic–pituitary–adrenal (HPA) axis activation in BACE1+/− transgenic mice following three different forms of behavioural stress.

Male BACE1+/− and wildtype (WT) mice (n=8/group) were exposed to an acute inescapable stress (elevated platform), acute escapable stress (elevated plus maze) or repeated inescapable stress (elevated plus maze for 10 days). Behaviour was assessed in the elevated plus-maze as the number of entries and time spent in the open and closed arms of the maze. Plasma corticosterone was measured by ELISA. Statistical analysis was performed using SPSS and significance set at P<0.05. BACE1+/− mice made significantly fewer entries into the open arm of the plus maze and made fewer total entries (closed and open arms) compared to WT animals. However, there was no significant difference in the total time spent in each arm. Plasma corticosterone was comparable at baseline. All animals showed a significant increase in corticosterone following exposure to acute or repeated stress. However, BACE1+/− mice showed a heightened response to the acute inescapable stressor (elevated platform). There was no effect of genotype on thymus or adrenal weight.

These findings demonstrate that BACE1+/− mice are more anxious and have an elevated stress response to an acute psychological stressor. These results show that reduction of BACE1 levels produce a maladaptive response in the HPA axis as reported for AD patients. This suggests that BACE1 may play a role in regulation of the HPA axis independent of Aβ production.

P113
Salivary cortisol monitoring in sheep and cows: evidence for acute activation of the hypothalamo–pituitary–adrenal (HPA) axis using two models of stress
Sarah Gibbs1, Emma Green1, Alun Ştedman1, Robert Abayasekara1, Derek Renshaw2 & Robert Fowkes1
1Royal Veterinary College, London, UK. 2University of Westminster, London, UK.

Inappropriate control of the hypothalamo-pituitary–adrenal (HPA) axis in production animals has serious consequences for animal welfare, and economic consequences in relation to food production. Fertility rates have declined as a consequence of farming practice and selective breeding, which may result in an increased susceptibility to disease and infection. Whilst precise monitoring of HPA status can be achieved through measurement of plasma cortisol levels, obtaining these samples from production animals can be time-consuming and invasive. Our current study was performed to determine the usefulness of salivary cortisol samples from sheep and cows, in assessing HPA activity. Two studies to model stress were employed; in the first, the stress response to shearing was determined in Suffolk x mule ewe lambs (n=10); in the second, the anxiety response following maternal separation in Holstein-Friesian or Angus-Friesian calves (n=8) was determined. In both studies, saliva samples were collected using either a swab or with a modified stomach tube and syringe. All samples were stored in salivettes and kept at ~20 °C, prior to centrifugation and assay for salivary cortisol (by ELISA). In the sheep studies, salivary cortisol dramatically increased within 1 h of shearing, compared with time-matched controls, and remained elevated for up to 6 h post-shearing. In the cow studies, salivary cortisol was also dramatically elevated in calves within 1 h of maternal separation; however, despite having a similar basal cortisol value, the stress response in calves allowed a longer period with their mothers prior to separation. The results from both studies suggest that salivary cortisol measurements in sheep and cows are effective at quantifying HPA activity in these models of restraint and psychosocial stress. Such an approach could be used to improve monitoring of husbandry practice in production animals.
The effects of recombinant human IGF1/IGFBP3 on body composition and physical fitness in recreational athletes
Nishan Guha1, Ioulietta Erotopkitou-Mulligan1, Simon Nevitt1, Michael Francis2, John Woodland3, Eryl Bassett4, Peter Sonksen1 & Richard Holt1
1University of Southampton School of Medicine, Southampton, UK; 2School of Mathematics, Statistics and Actuarial Science, University of Kent, Kent, UK.

Introduction
GH is widely abused by athletes for its anabolic and lipolytic properties. As the tests for detecting GH abuse develop further, it is possible that athletes will exploit IGF1 as an alternative or additional doping agent. There is currently no evidence to suggest that IGF1 administration improves athletic performance.

Objectives
To determine the effects of rhIGF1/rhIGFBP3 administration on body composition and physical fitness in recreational athletes. This study was part of a randomised, double-blind, placebo-controlled trial studying detection methods for IGF1 abuse.

Methods
The study received approval from the local ethics committee. Fifty-six recreational athletes (age 18–30 years, 30 males, 26 females) were randomly assigned to receive placebo, low dose rhIGF1/rhIGFBP3 complex (30 mg/day) or high dose rhIGF1/rhIGFBP3 complex (60 mg/day). Treatment was self-administered by s.c. injection for 28 consecutive days. Body composition (assessed by dual energy X-ray absorptiometry) and cardiorespiratory fitness, measured in terms of maximal oxygen uptake (VO2 max), were assessed before and immediately after treatment. Data from subjects in low and high dose treatment groups were combined and intra-individual changes were analysed using paired t-tests.

Results
There were no significant changes in body fat percentage or lean body mass in women or men after administration of rhIGF1/rhIGFBP3 complex. In both women and men, there were significant increases in VO2 max after treatment (P<0.001); HDL (mean increase 0.16±0.03 mmol/l, P<0.001) and LDL (mean increase 0.37±0.1 mmol/l, P<0.001). No changes in cholesterol/HDL ratio or fasting NEFA were observed. Fasting insulin and HOMA-IR decreased in both women and men treated with rhIGF1/rhIGFBP3; there was also a significant decrease in HbA1c in men (mean reduction 0.3±0.1%, P<0.001) but not in men.

Conclusions
Fasting triglycerides decrease in recreational athletes after the administration of rhIGF1/rhIGFBP3 for 28 days; these changes are associated with increased insulin sensitivity. RhIGF1/rhIGFBP3 appears to have a more pronounced effect on lipid and glucose homeostasis in women than in men.

Hypothalamic tanyocytes express nuclear receptor regulating enzymes in the absence of mRNA transcript
Kirsty Shearer, Patrick Stoney, Jemina Ransom, Gisela Heffer, Sandy Ross, Peter Morgan & Peter McCaffrey
University of Aberdeen, Aberdeen, UK.

Tanyocytes line the wall of the third ventricle sitting at the boundary between the cerebral spinal fluid (CSF) and the hypothalamus. Tanyocytes actively take up substances from the CSF using their villi-like apical projections that extend into the third ventricle. Retinol is one such compound and can be oxidized by these cells to retinoic acid, which can be released to regulate transcription in the hypothalamus via specific nuclear receptors. Two retinal dehydrogenases (RALDHs) required to catalyze this reaction, RALDH1 and RALDH2, are present in tanyocyte processes that infiltrate the hypothalamus. However, when mRNA from rat hypothalamic tissue is quantified for these enzymes only RALDH1 is present in tissue but both proteins are clearly present. The possible absence of RALDH2 mRNA transcript in tanyocytes suggests that the enzyme may be derived from an alternative source. Western blotting of CSF identified that RALDH2 is present in CSF and may, hypothetically, be taken up by tanyocytes. Thus CSF may potentially provide both retinol and the enzyme required to convert retinol to retinoic acid. One possible source of RALDH2 for the CSF is the choroid plexus, which produces the CSF including the proteins transthyretin and retinol binding protein that transport retinol. RALDH2 protein and transcript were detected in the choroid plexus by immunohistochemistry and in situ hybridization respectively. Interestingly, a second source of RALDH2 for the CSF is the meninges which, in the lateral ventricles, borders the hippocampus and is in direct contact with the CSF. RALDH2 transcript was detected in the meninges by in situ hybridization while protein was detected immunohistochemically in cytoplasmic vesicles. Other hypotheses for the source of RALDH2 are presently being tested.

The unique origins and tanyocyte subtype expression patterns of RALDH1 and RALDH2 in the hypothalamus suggest that retinoic acid generated by these two enzymes is required to regulate differing events.

Differential regulation of the neurotrophins, NFG and BDNF, and their receptors in the myometrium of women affected by adenomyosis
Anthony Taylor, Michael Hawes, Vijay Kalathy, Muna Abbas, Mohamed Mehasseb & Marwan Habiba
University of Leicester, Leicester, Leicestershire, UK.

Adenomyosis is an oestrogen-dependent uterine disease where ectopic, non-neoplastic endometrium is histologically observed within the myometrium. Its incidence ranges between 5 and 70% and although presenting symptoms; menorrhagia (40–50%), dysmenorrhoea (10–30%) and metrorrhagia (10–12%) are well known, its aetiopathology remains unclear. The neurotrophin, nerve growth factor (NGF), has been implicated in the aetiopathology of adenomyosis, especially in the tamoxifen-dosed neonatal CD-1 mouse, which develops severe adenomyosis through increased NGF expression. Preliminary data indicates that the neurotrophin system (ligands and receptors) are present in the human uterus, but have not been examined in adenomyosis.

In this study, immunohistochemistry and quantitative RT-PCR were used to examine NGF, BDNF, p75, trkA, trkB and trkC expression in human
adenomyotic myometrium. The effect of oestradiol and tamoxifen on NGF mRNA levels measured in control and adenomyotic myometrial cell cultures. Histomorphometric analyses of the immunohistochemical staining indicated an increase in NGF protein in the inner adenomyotic myometrium throughout the menstrual cycle, whilst trkC expression was unchanged. In the proliferative phase, trkB was significantly decreased throughout the myometrium and significantly increased in the secretory phase, whilst trkA showed a significant decrease only in the outer myometrium during the secretory phase; p<0.05. NGF transcript levels were significantly increased in adenomyotic inner myometrium, (1.5-fold; P<0.05; Student’s t-test) and significantly decreased in the outer myometrium, whilst BDNF, p75, trkB and trkC transcript levels were significantly decreased by 60, 90, 50 and 35%, respectively. Furthermore, NGF transcript levels were significantly (P<0.001; one-way ANOVA; n=12) reduced in both normal (4.5-fold) and adenomyotic (1.25-fold) cultures in response to oestradiol and to a lesser extent in both normal (2.6-fold) and adenomyotic (1.36-fold) cultures by tamoxifen.

These differences suggest a possible role for the neurotrophins and their receptors in the adenomyotic myometrium and in the aetiology/pathology of this common disease.

P118
Impact of synovial fibroblasts on adipose tissue
Ahkeb Hussain1, Rowan Hardy1, Pushpa Patel1, Mohammad Ahassan1, Andrew File2, Paul Stewart1 & Mark Cooper1
1The University of Birmingham, School of Clinical and Experimental Medicine, Birmingham, West Midlands, UK; 2The University of Birmingham, School of Immunity and Infection, Birmingham, West Midlands, UK.

Rheumatoid arthritis (RA) is associated with a loss of lean mass and a corresponding increase in fat mass. How fat accumulation in RA is linked to synovial inflammation is unknown. Wnts comprise a family of secreted glycoproteins that are crucial in regulating adipocyte proliferation, apoptosis and differentiation. Recently we demonstrated that endogenously generated glucocorticoids (GCs) alter the pattern of wnt secretion by synovial fibroblasts (SFs), favouring production of wnt inhibitors. Inhibition of wnt signalling in adipocytes promotes their differentiation through inhibition of PPARγ. In this study we have investigated whether wnt production by SFs could influence adipocyte differentiation and thus contribute to the increased fat accumulation seen in RA. Media conditioned for 24 h (CM) was collected from primary human SFs following 24 h pre-treatment with vehicle, TNFα or dexamethasone. 200 μl of CM was co-cultured with Chub-S7 human pre-adipocytes for 10 days – → differentiation media. Differentiation was assessed by Nile red staining followed by FACS analysis and expression of adipocyte differentiation genes G6PDH, 11β-HSD1, lipoprotein lipase (LPL) and leptin by real time RT-PCR in three separate experiments. Co-culture with un-stimulated CM did not induce adipocyte differentiation, as measured by lipid accumulation or gene expression. Significant increases in all markers of adipocyte differentiation were observed in response to TNFα treated CM (11β-HSD1, 3.6-fold; G6PDH, 18-fold; LPL, 30-fold; leptin, 15-fold; P<0.05). Similarly treatment with CM from SFs exposed to Dex significantly increased expression of adipocyte differentiation genes (G6PDH, 156-fold; LPL, 37-fold; Leptin, 5.4-fold; P<0.05). CM from cells previously exposed to TNFα or Dex resulted in increased lipid accumulation (3% ±0.11; 2.8% ±0.11 respectively; P<0.05) determined by FACS. These data suggest that when exposed to pro-inflammatory cytokines or GCs, SFs produce secreted factors, likely of the wnt family, that enhance adipocyte differentiation. These data provide a direct link between joint inflammation and abnormal fat accumulation.

P119
Cytokine profiling of pre-diabetic patients
Saket Gupta1,2, Ashwini Maratha1,2, Thusitha Gajanayake1,2, Jakub Siedienkin1,2, Anandan Natarajan1,2, Shu Hoashi1,2 & Sinead Miggin1,2
1Immune Signalling Laboratory, Institute of Immunology, National University of Ireland, Maynooth, Co. Kildare, Ireland; 2Midlands Regional Hospital, Mullingar and Royal College of Surgeons in Ireland, Mullingar, Co. Westmeath, Ireland.

Cytokine profiling of pre-diabetic patients
Society for Endocrinology BES 2011, Birmingham, UK

Diabetes, metabolism and cardiovascular
P120
Inflammatory markers are not strongly associated with the metabolic syndrome in type 2 DM
Anthoni Ogbora1,2 & Alfed Azanabor1,2
1Lagos State University Teaching Hospital, Lagos, Nigeria; 2Lagos University Teaching Hospital, Iddi-araba, Lagos, Nigeria.

Background
We sought to document the pattern of distribution of cytokines in patients with type 2 DM and compare cytokine levels between DM subjects with and without the Mets.

Methods
Out of 200 patients with type 2 DM and 100 healthy sex and aged matched controls were studied. Anthropometric indices, lipid parameters and cytokines levels which included interleukin 10 (IL10), tumor necrosis factor α (TNF), interferon γ (IFN) and C reactive protein (CRP) were determined. Continuous variables were compared between subjects with type 2 DM and the controls and also between DM patients with and without the Mets. The test statistics used included t-test, χ2, correlation coefficient and multiple regression analyses.

Results
The mean levels of all studied cytokines were significantly higher in the subjects with type 2 DM than in the control subjects. The mean cytokine levels were comparable in the DM subjects with and without the Mets and also comparable in obese DM and non-obese DM patients. Of the Mets defining criteria, waist circumference and TG were found to be significantly associated with only two cytokines, The correlation coefficient and multiple regression analyses.

Conclusions
Cytokine levels are higher in DM patients than in non DM subjects. However the cytokine levels are not strongly associated with the Mets. Limited correlations were found between each of the cytokines and the parameters of the Mets.

P121
Renal ischemia/reperfusion injury in type II DM: possible role of proinflammatory cytokines, apoptosis, and nitric oxide
Mahmoud Gabr1, Abdell-aziz Hussein1, Iman Sherif1, Sousou Ali1 & Hoda Mohamed2
1Faculty of Medicine, Mansoura, Egypt; 2Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

Background
Diabetes mellitus (DM) especially type II is a major health problem and diabetic nephropathy mediators such as IL6, TNFα and IL1b have been detected in the serum of diabetic patients. Herein, we investigated whether pro-inflammatory cytokines also exhibited perturbations in circulating pro-inflammatory cytokines. To this end, we recruited 42 healthy non diabetics and subjected them to 75 g OGTT after an overnight fast. We profiled the cytokines that are present in the serum of non diabetics (n=33, BMI 27.3±5.6, Hba1c 5.5±0.2%) and compared to pre-diabetic patients (n=9, BMI 33.2±8.5, Hba1c 6.5±0.3%) by multiplex cytokine profiling. Our data clearly show for the first time that levels of serum interferon-β (IFN-b) are significantly increased in pre-diabetic patients. In contrast, comparable levels of IL6, TNFα and IL1b are evident in the serum of pre-diabetic patients. Together, these data demonstrate that pre-diabetic patients exhibit perturbations in cytokine levels compared to normal individuals and support a role for these molecules in the disease progression to a diabetic pathology. Supported by HRB and SFI Ireland.

Table 1 Cytokine profiling of pre-diabetic patients.

<table>
<thead>
<tr>
<th>Cytokine (pg/ml)</th>
<th>Normal (mean±s.d.)</th>
<th>Pre-diabetic (mean±s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL6</td>
<td>1.14±0.59</td>
<td>1.59±0.59</td>
</tr>
<tr>
<td>TNFa</td>
<td>14.28±1.24</td>
<td>16.80±1.86</td>
</tr>
<tr>
<td>IL1b</td>
<td>0.84±0.41</td>
<td>0.91±0.69</td>
</tr>
<tr>
<td>IFNβ</td>
<td>1.29±1.2</td>
<td>5.54±2.09</td>
</tr>
</tbody>
</table>

CRP (g/l) 0.59 1.24
IL10 (pg/ml) 1.24 5.54
Interleukins 1.14 1.59
Tumor necrosis factor 1.24 1.86
Interferon 1.24 1.86
C reactive protein 0.59 1.59

Out of 200 patients with type 2 DM and 100 healthy sex and aged matched controls were studied. Anthropometric indices, lipid parameters and cytokines levels which included interleukin 10 (IL10), tumor necrosis factor α (TNF), interferon γ (IFN) and C reactive protein (CRP) were determined. Continuous variables were compared between subjects with type 2 DM and the controls and also between DM patients with and without the Mets. The test statistics used included t-test, χ2, correlation coefficient and multiple regression analyses.

Results
The mean levels of all studied cytokines were significantly higher in the subjects with type 2 DM than in the control subjects. The mean cytokine levels were comparable in the DM subjects with and without the Mets and also comparable in obese DM and non-obese DM patients. Of the Mets defining criteria, waist circumference and TG were found to be significantly associated with only two cytokines, The correlation coefficient and multiple regression analyses.

Conclusions
Cytokine levels are higher in DM patients than in non DM subjects. However the cytokine levels are not strongly associated with the Mets. Limited correlations were found between each of the cytokines and the parameters of the Mets.
Mechanisms behind this increased vulnerability not fully understood. The present study investigated the effect of acute ischemia for 45 min on proinflammatory cytokines, apoptotic markers, and nitric oxide (NO) in a rat model of type II diabetes.

Material and methods
Sixty male Sprague–Dawley rats were divided into 4 groups (% n = 15 each); Group I: normal rats, Group II: normal rats underwent left renal ischemia for 45 min, Group III: diabetic rats without renal ischemia, Group IV: diabetic rats underwent left renal ischemia for 45 min. Blood and kidney samples were taken 24 h after ischemia. Serum glucose, fructoseamine, creatinine, TNFα, as well as the expression of TGFβ, NFκB, iNOS, survivin, and Bcl2 in kidney tissue were measured.

Results
Type II DM caused significant increase in serum glucose, fructoseamine, creatinine, and TNFα and expression of TGFβ, NFκB and iNOS in renal tissue (P < 0.001). Also, DM caused significant increase in apoptotic cell death with increase in Bcl2 expression and decreased survivin in kidney. 45 min ischemia in diabetic rats caused more significant increase in serum TNFβ and expression of TGFβ, NFκB and iNOS (P < 0.001). Also; there was a positive correlation between blood glucose and TNFα, TGFβ, NFκB and iNOS with negative correlation with survivin (P < 0.01).

Conclusion
Type II DM render the kidney more susceptible to ischemic injury. Proinflammatory cytokines TNFα, TGFβ, and NFκB and iNOS as well as Bcl2 and survivin may contribute to the enhanced renal ischemic injury in type II DM. Also, hyperglycaemia may be involved in hypersensitivity of kidney to ischemic injury in DM.

---

P122
Adult patients with congenital adrenal hyperplasia have elevated blood pressure but otherwise a normal cardiovascular risk profile

Christianna Moom, Jeanne Margot Kroese, Fred Sweep, Ad Herms & Cees Tack

Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands.

Objective
Treatment with glucocorticoids and mineralocorticoids has changed congenital adrenal hyperplasia (CAH) from a fatal to a chronic lifelong disease. As a result of long-term treatment, including chronic (over) treatment with glucocorticoids, patients with CAH may develop an adverse cardiovascular risk profile. The objective of this study was to evaluate the cardiovascular risk profile of adult CAH patients.

Patients and measurements
In this case–control study the cardiovascular risk profile of 27 adult CAH patients and 27 controls, matched for age, gender and body mass index was evaluated by measuring ambulatory 24-h blood pressure, insulin sensitivity (HOMA-IR), lipid profiles, albuminuria and circulating cardiovascular risk markers (PAI-1, I PA, uPA, tPA, PAI-1 complex, hsCRP, adiponectin, IL6, IL18, and leptin).

Results
Twenty-four-hour systolic (126.3 mmHg ± 15.5 vs 124.8 mmHg ± 15.1 in controls, P = 0.019) and diastolic (76.4 mmHg ± 12.7 vs 73.5 mmHg ± 12.4 in controls, P = 0.001) blood pressure was significantly elevated in CAH patients compared to the control population. CAH patients had higher HDL cholesterol levels (P < 0.01), lower hsCRP levels (P = 0.03) and there was a trend toward elevated adiponectin levels compared to controls. Other cardiovascular risk factors were similar in both groups. Average BMI was high in CAH patients (27.2 ± 4.6 kg/m²). It was not possible to evaluate BMI as an individual cardiovascular risk marker because controls were matched for BMI.

Conclusion
Adult CAH patients have higher ambulatory blood pressure compared to age, gender and body mass matched controls, which may be related to glucocorticoid and mineralocorticoid treatment. Average body mass was high. Other cardiovascular risk markers did not differ, while HDL-cholesterol, hsCRP and adiponectin levels tended to be more favourable.

---

P123
The effects of Syzygium aromaticum derived oleic acid on glycogenic enzymes in streptozotocin induced-diabetic rats

Phakalelani Ngubane, Bubuya Masola & Cephas Musabayane

University of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa.

Diabetes mellitus is characterized by partial or total deficiency of insulin resulting in derangement of carbohydrate metabolism and a decrease in the activity of glycogenic enzymes resulting in depletion of liver and muscle glycogen. Accordingly, this study assessed the influence of OA on two key glycogenic enzymes of skeletal muscle and liver tissues in STZ-induced diabetic rats. We have, however, reported that the anti-hyperglycaemic effects of Syzygium aromaticum derived oleic acid (OA) in streptozotocin (STZ)-induced diabetic rats are mediated in part via increased hepatic glycogen synthesis. Hepatic and gastrocnemius muscle glycogen concentrations and activities of glucokinase (GK) and hexokinase (HK) of STZ-induced diabetic rats were measured after 5 weeks of twice daily treatment with OA (80 mg/kg, p.o.). Rats treated with deionised water (3 ml/kg, p.o.), or standard hypoglycaemic drugs (insulin, 200 μg/kg, s.c.; metformin, 500 mg/kg, p.o.) acted as untreated and treated positive controls, respectively. HK and GK activities were measured spectrophotometrically in reactions where the oxidation of glucose-6-phosphate formed was coupled to NADP⁺ reduction catalyzed by glucose-6-phosphate dehydrogenase. After 5 weeks STZ-induced diabetic rats exhibited decreased glycogen levels and low activities of glycogenic enzymes in muscle and hepatic tissues. OA administration increased the activity of glycogenic enzymes with concomitant restoration of muscle and hepatic glycogen concentrations to near normalcy. Interestingly, the combination of OA and insulin did not significantly alter the activities of HK and GK of STZ-induced diabetic rats suggesting that glycogen synthesis can also occur from precursors such as amino acids or fructose and lactate. Our data suggest that OA administration restores the activity of key glycogenic enzymes in the liver and skeletal muscle of STZ-induced diabetic rats to enhance glycogen synthesis to improve the glycemic status. The restoration of this principal glucose utilization pathway by OA will constitute a novel therapeutic strategy for diabetes treatment.

---

P124
Serum 25-hydroxy-vitamin D is associated with adiponectin and insulin resistance in diabetic Saudi adults

Nasser Al-Daghri1, Omran Al-Attal1, Majed Alokail1, Khalid Alkhafary1, Hossam Draz1 & Mario Clerici1,2

1King Saud University, Riyadh, Saudi Arabia; 2University of Milan, Milan, Italy.

Hypovitaminosis D is associated with an increased prevalence of diabetes mellitus type 2 (DM2) and the metabolic syndrome. The purpose of this study was to examine the association between 25-hydroxy-vitamin D (25-OH-VitD) levels and adipokines and other indices of insulin resistance in a Saudi population with DM2. One-hundred and fifty-five male and female Saudi adults aged 26–80 were randomly selected from the existing Biomarkers Screening in Riyadh Program (RIYADH Cohort). Subjects were clinically assessed, anthropometry was obtained and serum 25-OH-VitD, leptin, adiponectin, resistin, insulin, CRP, TNF-α, glucose, triglycerides, total cholesterol, LDL, and HDL concentrations were measured. Results showed a negative correlation between 25-OH-VitD and BMI, LDL and glucose and a positive correlation between 25-OH-VitD and adiponectin, which remained significant after controlling for BMI. Thus, 25-OH-VitD serum levels are negatively correlated with adiposity, insulin resistance, and LDL levels in Saudi patients with DM2. These results, together with the observed positive association between adiponectin and 25-OH-VitD suggest a role for this hormone as a link between 25-OH-VitD and insulin resistance and a possible beneficial effect of 25-OH-VitD on cardiovascular pathology.

---

P125
Endoplasmic reticulum oxidoreductases and insulin folding

Gautam Rajpal, Ming Liu & Peter Arvan

University of Michigan, Ann Arbor, Michigan, USA.

Proinsulin makes three evolutionarily-conserved disulfide bonds, two of which connect the insulin B and A chains, and one intrachain bond within the A chain. In Type II diabetes mellitus, increasing evidence suggests that impaired pancreatic β cell mass shows signs of endoplasmic reticulum (ER) stress and an increase in the presence of proinsulin with mispaired disulfide bonds. In addition, heterozygous expression of misfolded mutant proinsulin is known to cause autosomal dominant diabetes, resulting in a loss of β cell mass. ER oxidoreductases are involved in protein chaperone activity as well as redox reactions leading to formation of disulfide bonds of secretory proteins. The ER is a more oxidizing environment than that of the cytosol, at least in part due to the actions of Ero1 and Ero1 which become reduced by ER oxidoreductases. In turn, cargo proteins in the ER lumen transfer reducing equivalents to ER oxidoreductases, which results in formation of disulfide bonds within the ER.
secretory cargo. In endocrine pancreatic β cells, the major secretory cargo protein is proinsulin, the precursor in insulin biosynthesis. Thus, proinsulin is a major source for donating reducing equivalents to ER oxidoreductases, yet very little is known about the oxidoreductases that facilitate its proper disulfide bond formation within the ER. To begin to characterize the relationship between ER oxidoreductases and proinsulin within the ER of β cells, I have employed selective RNAi-mediated knock-down of ER oxidoreductases. Using anti-insulin immunoprecipitates from metabolically-labeled cells, I have used Tris-tricine-urea-SDS-PAGE to closely examine disulfide bond formation in endogenous proinsulin. By pinpointing the key ER oxidoreductases involved in forming proinsulin’s three native disulfide bonds, we can potentially manipulate these ER oxidoreductases to enhance proper proinsulin disulfide bond formation. In turn, this may alleviate ER stress, preventing β cell failure and the development of Type II diabetes mellitus.

P126

Does the effect of non-alcoholic fatty liver disease confer increased cardiovascular risk in patients with polycystic ovary syndrome? Allison Dawson¹, Thozhukat Sathyapalan², Eric Kilpatrick² & Stephen Atkin³

¹University of Hull, Hull, UK; ²Hull York Medical School, Hull, UK; ³Hull and East Yorkshire NHS Trust, Hull, UK.

Background

Polycystic ovary syndrome (PCOS) and non-alcoholic fatty liver disease (NAFLD) are both associated with the metabolic syndrome and are each associated with increased cardiovascular risk. Endothelial function, an early feature in the development of atherosclerosis, has been shown to be abnormal in both PCOS and NAFLD.

Method

The aim of this study was to determine if NAFLD conferred additional cardiovascular risk compared to PCOS alone.

Results

There was no difference between the PCOS-NAFLD and PCOS groups with respect to systolic (125.20 ± 12.93 vs 122.00 ± 9.50 P = 0.15) or diastolic (72.80 ± 12.93 vs 71.00 ± 5.90 P = 0.21) blood pressure. Cholesterol (4.67 ± 0.68 vs 4.51 ± 0.68 P = 0.68), HDL (1.13 ± 0.52 vs 1.09 ± 0.26 P = 0.60), LDL 2.96 ± 0.70 vs 2.89 ± 0.70 P = 0.93) and triglycerides (1.58 ± 0.68 vs 1.19 ± 0.43 P = 0.46) were no different between the two groups. The PCOS-NAFLD group had a higher body mass index (BMI) (45.72 ± 9.78 vs 37.47 ± 3.87 P = 0.03) with increased waist (128.60 ± 15.14 vs 113.54 ± 9.11 P = 0.01) and hip (153.85 ± 12.86 vs 123.35 ± 9.14 P = 0.01) circumference. There was no difference in RH-PAT between the PCOS-NAFLD and PCOS groups (1.91 ± 0.39 vs 1.80 ± 0.45 P = 0.38).

Conclusion

This study suggests that additional fatty liver disease in PCOS does not confer additional cardiovascular risk compared to PCOS alone, and despite the PCOS-NAFLD group having a higher BMI.

P128

Safety profile of Vildagliptin in clinical practice: 2-year data from 2 Tertiary hospitals

Mohammad Siddiqui¹, Mukul Gupta¹, Subhash Wangnoo¹ & Jamal Ahmad²

¹Apollo centre for Obesity, Diabetes and Endocrinology, Indraprastha Apollo Hospital, New Delhi, India; ²Centre for Diabetes and Metabolism, Aligarh Muslim University, Aligarh, Uttar Pradesh, India.

Background and aims

Proper assessment of long-term safety of newer antidiabetic agents (ADAs) is of prime importance as diabetes is a chronic disease requiring life-long management. The purpose of this analysis was to assess the safety of vildagliptin as monotherapy or as an add-on therapy to already existing ADAs (vildagliptin group) compared to non-vildagliptin group.

Materials and methods

Nine hundred and eighty-three patients in vildagliptin group and 1013 patients in non-vildagliptin group were included in the study drawn from the diabetes clinics. The dose of vildagliptin was either 50 or 100 mg/day, either as monotherapy or as an add-on therapy. Safety data included evaluation of clinical and laboratory adverse experiences (AEs).

Results

The incidence of drug-related AEs overall and discontinuations were higher in non-vildagliptin group, primarily due to hypoglycaemia in SUs and basal insulin-treated, and due to GI side effects, especially due to AGIs. For specific AEs reported more frequently in the vildagliptin group (with 95% CI), 4 AEs were higher in the vildagliptin group (generalized itching, non-specific bullous rash, small joint pain and dysaesthesia) and 6 were higher in ADAs group (nausea, diarrhoea, fatigue, headache, dizziness, and weight gain). There was no increased incidence of any infections, including urinary tract infections in vildagliptin group compared to other ADAs, slight but non-specific elevation of hepatic transaminases with vildagliptin monotherapy/add-on therapy, which resolved spontaneously.

Conclusion

Patients with type 2 diabetes tolerated vildagliptin as a monotherapy or an add-on therapy for nearly 2 years without any increased incidence of significant side effects.
olanzapine and clonazepam have the highest propensity to induce diabetes compared to other atypical antipsychotics. We present a case of a 37-year-old lady with advanced Huntington’s chorea who was admitted after general deterioration at home over 3 days, with loss of appetite, high temperature, worsening of chorei form movements, urinary frequency and reduced level of consciousness. On admission she was found to be in significant metabolic acidosis (pH 7.22), hyperglycaemia (glucose 73.3 mmol/l), renal failure and hypernaemia (Na+ 170). Inflammatory markers were raised (WCC 23.6, CRP 42) and her urine dipstick was positive for blood, protein, glucose, and ketones. She was treated for urinary sepsis with IV antibiotics, IV fluids and insulin sliding scale. There was no family history of diabetes. She was on olanzapine 20 mg once/day started 2 years prior to admission as well as sulpiride 200 mg mornings, and 600 mg evenings. She was discharged home on insulin (Mixtard 30) 34 units morning, and 16 units evening. Olanzapine and sulpiride were not stopped as these formed an important part in symptom control for her Huntington’s chorea.

Conclusion

Patients on atypical antipsychotics should be monitored for any signs and symptoms of hyperglycaemia and the complications associated with diabetes. Physicians must be made aware of the growing association between atypical antipsychotic agents and hyperglycaemia.

### P130

**Cabergoline prevents weight gain in patients evaluated for hyperprolactinaemia**

Martin Whyte1, Riaz Aziz1, Sesha Pramodh2 & Simon Aylin1

1King’s College Hospital, London, UK; 2Yeovil Hospital, London, UK.

**Introduction**

Food reward stimuli elevate dopamine levels in brain reward circuits. Decreased dopaminergic signalling may be involved in pathophysiological processes leading to obesity and D2 receptor antagonists (antipsychotics) are associated with a higher risk of obesity. One study has demonstrated an association between the use of a dopamine D2 agonist (bromocriptine) and weight-loss in patients with prolactinoma. We have investigated the effect of cabergoline on body weight in a population presenting with hyperprolactinaemia.

**Methods**

One hundred medical records were studied. All patients had been evaluated for prolactin excess with a cannulated prolactin study. Macroprolactinaemia was excluded in all samples. Patients with panhypopituitary or previously treated with another dopamine agonist were excluded. Data was also unavailable in 3 patients, leaving 89 patients. Weight and cabergoline dose was recorded at each visit.

**Results**

At Visit 1, prolactin was 1614.4 ± 447.8 mU/l in cabergoline group (CG) and 534.1 ± 75.8 mU/l in controls (P < 0.001). Body weight and BMI at the first visit was 72.5 ± 13.7 kg and 27.0 ± 6.5 kg/m^2 in CG and 70.6 ± 9.0 kg and 26.9 ± 1.1 kg/m^2 in controls (P = NS). Cabergoline dose was 493.2 ± 15.4 μg/week.

**Conclusion**

Cabergoline is unlikely to be considered as a useful weight loss agent, but in this study it was associated with weight stability compared to the weight gain that occurred amongst individuals who did not start dopamine agonist treatment.
**P133**

**Plasma Annexin A1 (AnxA1) is inversely correlated with waist to hip ratio in healthy males**

Anna Kosicka-Knox¹, Adam Cunliffe², Richard Mackenzie¹, Rod Flower³ & Derek Rentshaw¹

¹University of Westminster, London, UK; ²London South Bank University, London, UK; ³Bart’s and The London, London, UK.

The escalating public health problem represented by obesity has spurred multi-disciplinary research into adipose tissue and importantly, the molecular biology of the adipocyte. Recent studies suggest that obesity related metabolic data is characterised by chronic mild inflammation from fat tissue leading to dysregulation in the pro/anti-inflammatory systemic balance. Adipokines and pro-inflammatory markers are implicated in insulin insensitivity, blood glucose dysregulation, inflammation and atherosclerosis. As a result, circulating levels of adipokines and pro-inflammatory markers may be valuable biomarkers in identifying future risk of disease states associated with obesity. Whilst there is a considerable amount of research into the characterisation of adipokines and pro-inflammatory cytokines, the anti-inflammatory adipokines warrant further exploration. AnxA1, is an endogenous glucocorticoid regulated protein, which mediates systemic anti-inflammatory processes. The expression of AnxA1 with increased adiposity has not been investigated to date. However, given the balance that exists between pro and anti-inflammatory substances, it seems plausible that AnxA1 may be altered in individuals with increasing adiposity.

We recruited 94 healthy males for an in vivo study measuring both metabolic and anthropometric parameters. Plasma AnxA1 levels were correlated with levels of adiposity and distribution of fat, body mass index (BMI), waist to hip ratio, adipokynes, blood cholesterol, glucose and blood pressure. AnxA1 was measured using an in-house sandwich ELISA. Ethical approval was granted by the University of Westminster (08/09/22).

Our results show that plasma AnxA1 protein is significantly inversely correlated with waist to hip ratio (P<0.03) in healthy male subjects suggesting that as central fat mass increases the concentration of plasma AnxA1 decreases. AnxA1 could potentially represent a (fat) depot specific biomarker whose decline with increasing central adiposity may relate to the phenomena of increasing systemic inflammation and associated disease risk.

**P134**

**Characterisation of IGFBP1 molecular forms in healthy persons and patients with diabetes mellitus type 2 or hypoglycemia**

Dragana Lagundzin & Olgica Nedic
Institute for the Application of Nuclear Energy-INEP, 11080 Belgrade, Serbia.

Insulin-like growth factors (IGFs) and their binding proteins (IGFBPs) are involved in the regulation of glucose metabolism and metabolic disorders. IGFBP1 is the only IGF-binding protein that shows diurnal variation and is inversely correlated to the insulin level. IGFBP1 exists as non-phosphorylated protein and several phosphorylforms. Phosphorylation affects the affinity of IGFBP1 for IGFs and, therefore, the ability to regulate IGF action. In the circulation IGFBP1 is associated with τ2-macroglubulin (τ2M) in a complex that may have an important role in controlling IGF action by regulating the amount of free IGFBP1. IGFBP1 was found to form multimers as well, which have low affinity for IGFs. The aim of this work was to investigate the kinetics of the alteration of IGFBP1 concentration during OGTT, together with the molecular distribution of IGFBP1, in healthy persons, patients with diabetes mellitus type 2 (DM2) or hypoglycemia (HG).

It was found that IGFBP1 concentration significantly decreased 2 h after glucose intake in healthy and hypoglycemic persons, whereas in patients with diabetes a reduction in IGFBP1 became significant already after the first hour. The pattern of IGFBP1 phosphorylation and oligomerisation has been investigated using immunoaffinity chromatography with immobilised anti-τ2M antibodies, SDS-PAGE and SELDI-technology. The relative ratio of IGFBP1 isoforms seemed to be the same in all three study groups, but the groups differed in respect to oligomer formation (90–100 kDa). There were three oligomeric forms detected in the circulation of DM2 patients, compared to one in HG patients and two in healthy persons. Oligomers had characteristic masses in samples from three study groups. These results suggest a possible greater involvement of IGF system in glucose/insulin metabolism in patients with diabetes mellitus type 2, than in healthy persons and patients with hypoglycemia.

**P135**

**The Megalin-Cubulin receptor-mediated endocytic pathway is impaired in Dent’s disease renal proximal tubule cell-lines**

Caroline Gorvin¹, Martijn Wilmer², Nellie Loh¹, Sian Pirel³, Brian Harding¹, Lambertus van den Heuvel¹, Elena Levchenco² & Rajesh Thakker¹

¹Academic Endocrine Unit, O Chorem University, Oxford, Oxford, UK; ²Laboratory of Pediatrics and Neurology (656), Radboud University Nijmegen Medical Center, PO Box 9101, 6500 HB, Nijmegen, The Netherlands.

Receptor-mediated endocytosis (RME), involving megalin and cubulin, mediates renal proximal-tubular reabsorption of glucose, proteins and hormones including insulin, parathyroid-hormone and vitamin D. RME disruption occurs in Dent’s disease patients with mutations of the chloride/proton antiporter, CLC-5, who suffer from low-molecular-weight proteinuria, hypercalcaemia, nephrolithiasis and renal failure. To further investigate the RME role of CLC-5 we established conditionally immortalized proximal-tubular epithelial cell-lines (ciPTECs) from three patients with CLC-5 mutations (30tnHis, Arg637Stop and del132-241). Clonal populations of urinary cells were established by selection of aminopeptidase-N-positive cells using fluorescence-activated cell sorting, and characterised by flow cytometry, RT-PCR, western blot and confocal macroscopy to establish expression of ATP-binding cassette sub-family C member 4 (ABC4), Aquaporin-1, dipeptidyl peptidase-4 (DPPIV/CD26) and P-glycoprotein to demonstrate their proximal-tubular origin. Endocytosis was investigated by fluorescent-labelled albumin and transferrin uptake assays with and without 10-fold excess of unlabelled albumin or transferrin, and compared to two control ciPTECs. Confocal microscopy using the tight junction marker ZO-1, and EB1 which is specific for the plus-end of microtubules, were used to demonstrate that the ciPTECs polarised. Fluorescent-albumin-uptake in the three Dent’s disease ciPTECs was significantly decreased to 20–65% of control values (P<0.02). In ciPTECs with the 30tnHis and Arg637Stop, albumin-uptake was demonstrated to be further reduced by competition with unlabelled albumin and transferrin (13.23±3.01% and 16.44±7.45%, respectively for 30tnHis (P<0.02); and 36.12±6.62% and 43.72±3.54%, respectively for Arg637Stop (P<0.05) when compared to uptake without competition), thereby confirming the specificity of the albumin uptake by the megalin-cubulin RME pathway in these ciPTECs. Furthermore, transferrin-uptake was similarly reduced in the ciPTECs with CLC-5 mutations, consistent with impairment of CLC-5-megalin-cubulin RME in these Dent’s disease ciPTECs. Thus, these Dent’s disease ciPTECs which have defective RME, will help in elucidating the mechanisms involved in renal proximal-tubular reabsorption of solutes.

**P136**

**Influence of metabolic decompensation in children with diabetes mellitus type 1 on changes of QT interval**

Galina Meraai & Angelika Solntseva
First Department of Childhood Diseases, Belarusian State Medical University, Minsk, Belarus.

The basic manifestations of dystrophic changes in heart of children suffering form diabetes mellitus type 1 (DM1) are disturbances of repolarization and depolarization processes, including QT and QTc intervals prolongation. Research objective: to evaluate indices of QT and QTc in children with DM1, to reveal interrelation of the observed changes with the duration of disease, age, sex, levels of cholesterol (CH) and glycemic, BMI, blood pressure, pulse rate.

**P137**

**Influence of metabolic decompensation in children with diabetes mellitus type 1 on changes of QT interval**

Galina Meraai & Angelika Solntseva
First Department of Childhood Diseases, Belarusian State Medical University, Minsk, Belarus.

The basic manifestations of dystrophic changes in heart of children suffering from diabetes mellitus type 1 (DM1) are disturbances of repolarization and depolarization processes, including QT and QTc intervals prolongation. Research objective: to evaluate indices of QT and QTc in children with DM1, to reveal interrelation of the observed changes with the duration of disease, age, sex, levels of cholesterol (CH) and glycemic, BMI, blood pressure, pulse rate.
The increase of QT and QTc is noticed in children with DM1. Age, level of Conclusions established. duration of the disease and values of systolic and diastolic blood pressure was not P 0.0005). Values of QTc in girls with DM1 are higher than that values in boys (425.36 ± 14.8 ms versus 416.24 ± 14.8 ms, P < 0.002). 21.59% of girls and 15.79% of boys with DM1 have QTc > 440 ms. At the same time values of fructozamine level are higher in boys than in girls (412.88 ± 11.35 and 377.66 ± 8.76 mmol/l respectively, P < 0.0001). The difference in pulse rate between girls and boys is insignificant for the group studied. Connection of QTc of fructozamine level are higher in boys than in girls (412.88 ± 11.35 and 377.66 ± 8.76 mmol/l respectively, P < 0.0001). Reliable correlation between QTc and values of BMI, level of CH, duration of the disease and values of systolic and diastolic blood pressure was not established.

Conclusions The increase of QT and QTc is noticed in children with DM1. Age, level of glycemie, pulse rate and sex are the factors defining values of QTc.

P138
Hypogonadism with subsequent multi-organ involvement: a mystery solved
Cynthia Mohandas1, Dennis Barnes2, Derek Harrington1 & Masud Haq1
1University Hospital Lewisham, Lewisham, UK; 2Kent and Sussex Hospital, Royal Tunbridge Wells, UK.

A 53-year-old gentleman was seen following a recent diagnosis of type 2 diabetes in May 2009. He had suffered a subarachnoid haemorrhage in 1993 and remained under the local tertiary centre after developing secondary hypogonadism treated with testosterone replacement. The cause had never been established.

The patient had previously been diagnosed with seronegative HLA B27 arthropathy and in December 2008 was admitted with acute cardiac failure and atrial fibrillation. An echocardiogram demonstrated a moderately dilated right and left heart, global left ventricular hypokinesia, an ejection fraction of 20% consistent with dilated cardiomyopathy. He responded well to medical therapy. He had mildly abnormal liver function tests in the past but his AST level was twice the upper limit of normal in May 2009. Subsequent iron studies revealed a transferrin saturation of 90% and ferritin of 8989 μg/l. Testosterone replacement was therefore discontinued.

Hepatitis screen and liver autoantibodies proved negative. AFP level was <2. Abdominal ultrasound confirmed 23 cm hepatomegaly but no splenomegaly. The patient was confirmed to have hereditary haemochromatosis (codon 63-HH, codon 282-YY).

Baseline tests revealed: F.T4 12.5 µmol/l, TSH of 0.6 mIU/l; 9am cortisol 454 nmol/l; prolactin 101 µIU/l; testosterone 0.2 µmol/l, LH <0.1 IU/l, FSH <0.2 IU/l; IGF1 7.1 mmol/l and GH 1.26 µg/l. A TRH test was normal but a glucagon test confirmed GH deficiency with normal cortisol response. An MRI did not demonstrate pituitary enhancement but cardiac MRI has shown a grossly dilated LV with globally impaired function and severe myocardial iron loading. Ferritin levels have fallen to 3203 µg/l with regular venesection and he remains under annual surveillance by the gastroenterologists. Hereditary haemochromatosis is an autosomal recessive disorder characterised by excess iron deposition especially in the liver, heart, pancreas, and pituitary. This case is an excellent example of why this diagnosis should be excluded in patients with unexplained cardiac failure or hypogonadism.

P139
Pomegranate juice consumption influences urinary glucocorticoids, attenuates blood pressure and exercise-induced oxidative stress in healthy volunteers
Catherine Tsang, Gillian Wood & Emad Al-Dujaili
Queen Margaret University, Edinburgh, Scotland. UK.

Background and aim Antioxidants have been postulated to exert beneficial effects on cardiovascular and neurodegenerative diseases by neutralizing reactive oxygen species (ROS).

Exercise and metabolic processes are known to produce ROS. Pomegranates are rich in polyphenolic antioxidants. The aim of this study is to investigate the effects of pomegranate pure juice consumption on blood pressure, lipid peroxidation and urinary glucocorticoid levels before and after a moderate exercise bout.

Methods A randomized placebo controlled 2-arm study was conducted. Participants (2 groups of 10 each) attended two 30 min treadmill exercise sessions (50% Wmax); pre and one week post pomegranate juice (500 ml/day containing 1685 mg total phenolics/l) or water consumption. 24 h urine samples were collected and blood pressure monitored before and after each session. Urinary lipid peroxidation levels (TBARS), free cortisol and cortisone levels were determined in all urine samples using in house ELISA methods.

Results Pomegranate juice consumption was found to significantly decrease systolic blood pressure (pre-exercise: 141.2 ± 7.6 to 136.1 ± 7.3, P = 0.03 and post-exercise:156.4 ± 17.5 to 149.5 ± 10.2 mmHg, P = 0.04), diastolic blood pressure (90.9 ± 11.6 to 87.1 ± 8.7, P = 0.04 and 102.6 ± 23.9 to 94.6 ± 20.4 mmHg, P = 0.05) and TBARS levels (0.312 ± 0.106 to 0.264 ± 0.098 MDA mM/l, P = 0.035). There was no significant change in lipid peroxidation or blood pressure for subjects consuming water. Urinary free cortisol was reduced from 39.1 ± 26.6 to 26.4 ± 16.5 mmol/24 h (P=0.064), however there was a statistically significant increase in urinary free cortisone (28.1 ± 20.4 to 51.9 ± 45.1 mmol/24 h, P = 0.045), and decrease in free cortisol/cortisone ratio (1.81 ± 1.24 to 0.82 ± 0.56, P = 0.009) following one week of pomegranate juice intake.

Conclusions Our results suggest that pomegranate juice seems to exert beneficial effects in reducing blood pressure pre/post exercise and lipid peroxidation levels due to exercise-induced oxidative stress. The reduction in blood pressure could presumably be due to the inhibition of iNOS/HS1 activity as evidenced by the reduction in cortisol/cortisone ratio or other mechanisms yet to be investigated.
Audit of short synacthen tests in patients with type 2 diabetes mellitus
Logan Manikam1, Nadia Othonos1, Harit Buch1 & Rousseau Gama1,2
1New Cross Hospital, Wolverhampton, UK; 2Wolverhampton University, Wolverhampton, UK.

Background and objective
Addison’s disease occurs more frequently in patients with type 1 diabetes mellitus (T1DM) as part of the autoimmune polyendocrine syndromes. There is, however, no such association between adrenal failure and type 2 diabetes mellitus (T2DM).

We, therefore, retrospectively audited referrals for short synacthen tests (SST) on patients with T2DM.

Methodology
Seven years retrospective study of indications for and results of SST on patients with T2DM referred for exclusion of adrenal failure. A normal SST was defined as a serum cortisol increase of >200 nmol/l over baseline and peak serum cortisol response >550 nmol/l.

Results
There were 89 referrals SSTs in patients with T2DM. Recurrent hypoglycaemia was the sole indication for a SST in 55 (61.8%) patients and in 4 (4.5%) patients in combination with weight loss (n=3), hypopituitarism (n=1) and hyponatraemia (n=1), hypogonadotrophic hypogonadism (n=1) and dizziness without hypotension (n=1). Seventeen (19.0%) SSTs were performed on patients with known or suspected hypothalamic–pituitary–adrenal axis disorder including three on long-term steroids. The remaining 12 (13.5%) SSTs were requested because of weight loss (n=3), hyperkalaemia (n=3), hyponatraemia (n=1), postural hypotension (n=3), tiredness (n=1), reduction in insulin requirements (n=1) and to assess adrenal reserve prior to starting thyroxine (n=1). Three (3.4%) patients had suboptimal cortisol responses to synacthen, all of who were on long-term steroid therapy.

Discussion
Addison’s disease occurs more frequently only in T1DM and not T2DM. It is recommended that patients with T1DM with unexplained recurrent hypoglycaemia be screened for Addison’s disease. This, perhaps, has been inadvertently extrapolated to T2DM since the commonest indication for SST in patients with T2DM was recurrent hypoglycaemia but all had normal SSTs.

Conclusion
Recurrent hypoglycaemia, in the absence of other features of adrenal failure, is not an indication for a short synacthen test in patients with T2DM.

Retinal screening in pregnant women with diabetes: ‘are we doing enough’
Gideon Mlawa, Richard Holt, Mathew Coleman, Christina Rennie & Roger Smith
Southampton General Hospital, Southampton, UK.

Introduction
NICE recommends that pregnant women with pre-existing diabetes should be offered retinal assessment in the 1st and 3rd trimester by digital retinal imaging. Where retinopathy is already present, an additional assessment should be made in the 2nd trimester.

Objectives
To assess the feasibility of retinal screening in pregnancy by a community based mobile retinal screening programme (MRSP).

Methods
A retrospective audit of 58 pregnant women with pre-existing diabetes who attended the diabetes antenatal clinic between 2008 and 2009 was performed. The hospital notes and MRSP database were examined to assess whether the women were registered with MRSP, were offered a screening appointment and whether they attended at the appropriate time.

Results
Fifty-six patients were sent appointments offering retinal screening. 41 patients were screened in the 1st trimester or at first contact and again during the 3rd trimester. In addition all 8 patients with pre-existing retinopathy were screened in the 2nd trimesters. Five patients did not attend the appointment despite reminders. Their GP’s were informed and direct fundoscopy was undertaken in clinic. One woman was already receiving care from the eye unit. Seven women lived outside our catchment area and may have received screening elsewhere. Four women were not registered with the programme.

Conclusion
At least 72% of women received eye care as recommended by NICE. 9% did not attend despite reminders and 7% were not registered with the programme. Further work is needed to identify the patient and systematic barriers that prevented screening in these women.
Obesity is associated with metabolic and vascular dysfunction. Many models have shown insulin resistance reduces endothelium-dependent vasodilation but this is also seen in obese subjects with normal glucose tolerance. There is also evidence of increased response to vascular injury in obese animals, with mechanisms underpinning this not fully understood. This study used a mouse model of diet-induced obesity (DIO) to address the hypothesis that obesity causes metabolic dysfunction, inhibition of endothelium-dependent relaxation and increased neointimal proliferation following wire-induced injury.

Male C57Bl/6 mice (5 weeks old) were fed obesogenic or control diets (8 weeks) and then underwent metabolic testing, including blood pressure measurement by tail cuff plethysmography. They were then either killed and femoral arteries collected for function analysis (n=7/group), or subjected to femoral artery luminal wire injury surgery (N=5 CON 7 DIO/group) and killed 4 weeks post injury. Injured vessels were excised and lesion volume assessed using 3-dimensional optical projection tomography (OPT) and histology.

DIO mice were heavier than controls (P<0.01), had higher circulating cholesterol and triglyceride levels and were hyperglycaemic and hyperinsulinaemic (DIO 1.39±0.16 vs CON 0.52±0.08 mmol/l P<0.001) on fasting and following a glucose load (repeated measures ANOVA; glucose P<0.001, insulin P<0.05). Development of obesity did not alter blood pressure (DIO 119±4 vs CON 121±3 mmHg), agonist-mediated contraction or endothelium-dependent or -independent relaxation of isolated femoral arteries. Similarly, DIO had no effect on the size of neointimal lesions following femoral artery injury whether analysed using OPT (Lesion volume; DIO 29.9±9.0 versus CON 38.2±7.1%), or histology (maximum cross sectional area; CON 58.3±7.9 versus DIO 53.8±11.5%). Thus, in young mice, obesity-induced alterations in glucose/insulin and lipid metabolism occur in the absence of vascular dysfunction. More prolonged exposure to diet-induced obesity or metabolic abnormalities may induce vascular changes, though this remains to be explored.

Conclusion
Postprandial hypertriglyceridaemia is higher in patients with PCOS compared to normal women and could potentially contribute to their higher cardiovascular risk.

Abstract withdrawn.

Patterns of food consumption have a profound influence on hormone rhythmicity and fat storage, but until now only crude manipulations of food availability have been possible in rodents. We have used a CLAMS-based system in conjunction with automated serial blood sampling to investigate the effect of continuous feeding on ghrelin secretion and adiposity. Six-week old male Sprague-Dawley rats (n=6) were housed in metabolic cages and ad-libitum fed with standard chow for 3 weeks (12 h light:12 h darkness; lights on at 0600 h). A cohort of age- and weight-matched rats (n=4) were housed in CLAMS cages in the same room and subjected to paired, continuous nocturnal feeding (1/24th of the total daily food intake of ad-libitum-fed rats being available every 30 min of the 12 h dark phase) for 3 weeks. During week 3 jugular vein catheters were introduced under isofluorana anaesthesia and automated hourly blood sampling initiated after 48 h recovery. Continuously fed rats consumed only 96% of their total daily allowance (P<0.01), but body weight (BW) was not significantly reduced. The feeding-associated suppression of circulating octanoylated ghrelin in the early dark phase was significantly delayed in continuously fed rats: plasma concentrations at 1800, 1900 and 2400 h being 4.1, 2.7- and 3.3-fold lower in ad-libitum-fed rats. Although no-anus length and pituitary weight were unaffected by continuous feeding, femoral length was reduced by 2% (P<0.05) and proportionate liver weight increased by 6% (P<0.05). Proportionate abdominal fat pad weights were not significantly affected by continuous feeding, but when corrected for cumulative food intake, the retroperitoneal depot (which is most sensitive to ghrelin exposure) showed elevated lipid storage efficiency (increased by 24%; P<0.05). Thus, continuous feeding fails to induce the post-prandial suppression of ghrelin secretion and predisposes rats to increased fat accumulation, suggesting that longer-term grazing may be obesogenic without increasing caloric intake.

Conclusion
Continuous feeding in rats: a novel paradigm for inducing hypocaloric obesity?
Bradley Arms-Williams & Timothy Wells
School of Biosciences, Cardiff University, Cardiff, UK.

Patterns of food consumption have a profound influence on hormone rhythmicity and fat storage, but until now only crude manipulations of food availability have been possible in rodents. We have used a CLAMS-based system in conjunction with automated serial blood sampling to investigate the effect of continuous feeding on ghrelin secretion and adiposity. Six-week old male Sprague-Dawley rats (n=6) were housed in metabolic cages and ad-libitum fed with standard chow for 3 weeks (12 h light:12 h darkness; lights on at 0600 h). A cohort of age- and weight-matched rats (n=4) were housed in CLAMS cages in the same room and subjected to paired, continuous nocturnal feeding (1/24th of the total daily food intake of ad-libitum-fed rats being available every 30 min of the 12 h dark phase) for 3 weeks. During week 3 jugular vein catheters were introduced under isofluorana anaesthesia and automated hourly blood sampling initiated after 48 h recovery. Continuously fed rats consumed only 96% of their total daily allowance (P<0.01), but body weight (BW) was not significantly reduced. The feeding-associated suppression of circulating octanoylated ghrelin in the early dark phase was significantly delayed in continuously fed rats: plasma concentrations at 1800, 1900 and 2400 h being 4.1, 2.7- and 3.3-fold lower in ad-libitum-fed rats. Although no-anus length and pituitary weight were unaffected by continuous feeding, femoral length was reduced by 2% (P<0.05) and proportionate liver weight increased by 6% (P<0.05). Proportionate abdominal fat pad weights were not significantly affected by continuous feeding, but when corrected for cumulative food intake, the retroperitoneal depot (which is most sensitive to ghrelin exposure) showed elevated lipid storage efficiency (increased by 24%; P<0.05). Thus, continuous feeding fails to induce the post-prandial suppression of ghrelin secretion and predisposes rats to increased fat accumulation, suggesting that longer-term grazing may be obesogenic without increasing caloric intake.

Conclusion
Continuous feeding in rats: a novel paradigm for inducing hypocaloric obesity?
Bradley Arms-Williams & Timothy Wells
School of Biosciences, Cardiff University, Cardiff, UK.

Patterns of food consumption have a profound influence on hormone rhythmicity and fat storage, but until now only crude manipulations of food availability have been possible in rodents. We have used a CLAMS-based system in conjunction with automated serial blood sampling to investigate the effect of continuous feeding on ghrelin secretion and adiposity. Six-week old male Sprague-Dawley rats (n=6) were housed in metabolic cages and ad-libitum fed with standard chow for 3 weeks (12 h light:12 h darkness; lights on at 0600 h). A cohort of age- and weight-matched rats (n=4) were housed in CLAMS cages in the same room and subjected to paired, continuous nocturnal feeding (1/24th of the total daily food intake of ad-libitum-fed rats being available every 30 min of the 12 h dark phase) for 3 weeks. During week 3 jugular vein catheters were introduced under isofluorana anaesthesia and automated hourly blood sampling initiated after 48 h recovery. Continuously fed rats consumed only 96% of their total daily allowance (P<0.01), but body weight (BW) was not significantly reduced. The feeding-associated suppression of circulating octanoylated ghrelin in the early dark phase was significantly delayed in continuously fed rats: plasma concentrations at 1800, 1900 and 2400 h being 4.1, 2.7- and 3.3-fold lower in ad-libitum-fed rats. Although no-anus length and pituitary weight were unaffected by continuous feeding, femoral length was reduced by 2% (P<0.05) and proportionate liver weight increased by 6% (P<0.05). Proportionate abdominal fat pad weights were not significantly affected by continuous feeding, but when corrected for cumulative food intake, the retroperitoneal depot (which is most sensitive to ghrelin exposure) showed elevated lipid storage efficiency (increased by 24%; P<0.05). Thus, continuous feeding fails to induce the post-prandial suppression of ghrelin secretion and predisposes rats to increased fat accumulation, suggesting that longer-term grazing may be obesogenic without increasing caloric intake.
Method
Ten patients with a body mass index (BMI) >40 kg/m² were recruited and followed up for 12 months after surgery. Fasting blood samples were taken before surgery and at 3 monthly intervals after surgery. Endothelial function (RH-PAT) was also determined at these time points using peripheral arterial tonometry (endoPAT2000) technique. A 75 g oral glucose tolerance test was performed at the start of the study to exclude diabetes.

Results
BMI reduced from 49.47 ± 7.54 kg/m² pre surgery to 33.32 ± 5.21 (P < 0.001) 12 months after surgery. Fasting glucose levels reduced from 5.26 ± 0.78 mmol/l to 4.56 ± 0.33 (P = 0.02), C-reactive protein (CRP) from 7.22 ± 7.81 mmol/l to 2.41 ± 4.1 (P = 0.039), Systolic blood pressure reduced from 129.64 ± 16.18 mmHg to 114.22 ± 10.71 (P = 0.005) and diastolic blood pressure reduced from 76.55 ± 14.24 mmHg to 72.56 ± 7.50 (P = 0.95). RH-PAT improved from 1.80 ± 0.39 to 2.01 ± 0.54 (P = 0.035).

Conclusion
Roux-en-Y surgery in patients with normal glucose tolerance improved metabolic risk. Thus is shown by a reduction in the inflammatory markers CRP, a reduction in systolic and diastolic blood pressure and also improved endothelial function 12 months after surgery.

P150
The prevalence of non alcoholic fatty liver disease in GH deficiency and the effect of GH replacement
Chris Gardner1, Andrew Irwin1, Francis Joseph2, Chris Wong3, Val Adams2, Christina Daousi4,5, Graham Kemp1 & Daniel Cuthbertson1,2
1Aintree University Hospital NHS Foundation Trust, Liverpool, UK; 2Department of Human Metabolism, University of Liverpool, Liverpool, UK.

Background
Non-alcoholic fatty liver disease (NAFLD) is reported to be more prevalent in patients with GH deficiency (GHD) than in the general population. Case control studies have not however been undertaken. Recognition of NAFLD is important due to its association with cardiovascular disease and chronic liver disease.

Aims
To determine i) the prevalence of NAFLD in patients with severe GHD compared to age and BMI-matched controls, and, ii) the effect of 6 months GH replacement (GHR) on liver fat.

Patients and methods
Twelve patients (7 males) with GHD for >12 months (Peak GH <3 μg/l) on glucose stimulation test) and 12 controls matched for age, gender and BMI were studied. GHD patients were studied before and 6 months after initiation of GHR.

Ethics committee approval was obtained.

Results
Values are quoted as median (range). Age of patients was 44.5 (35.63) years versus controls 48.5 (33.66) years (P = 0.68). Patient BMI was 30.8 kg/m² (22.4, 45.3) versus controls 31.7 kg/m² (24.3, 44.3). IGFI was significantly lower in the patient group, (11.5 vs 16 mmol/l P = 0.03). There was no significant difference in ALT, AST, yGT, cholesterol, HDL, LDL or percentage IHCL. (3.6% (0.2, 44.8) patients versus 6.6% (0.5, 32.1) controls, P = 0.68). 5 patients and 6 controls had IHCL >5.5%. Of the patients with elevated IHCL, 4 commenced GH. Liver fat reduced from 28.1% (20.4, 44.8) pre treatment to 16.3% (5.5, 20.6) post treatment (P = 0.06).

Conclusions
Patients with GHD do not have an increased prevalence of NAFLD compared to matched healthy controls. GH replacement may reduce liver fat in patients with GHD. This is significant as patients with GHD have elevated cardiovascular risk.

P151
Serum erythropoietin levels in patients with acute myocardial infarction
Elham Islam, Iman Zaki, Mona Abdelsalam, Ahmed Abdelsalam, Samya Eltohamy & Ahmed Morsi
Ain Shams University, Cairo, Egypt.

Background
Mortality and morbidity after acute myocardial infarction (AMI) remain high even when myocardial reperfusion is successful. Thus, additional approaches are warranted. Erythropoietin (EPO) was found to be a cytoprotective molecule that might represent a novel strategy to limit the infarct size.

Aim of the work
To investigate serum erythropoietin level and its relation to infarct size in Egyptian patients with AMI.

Methods
We studied 20 patients with AMI who underwent PCI within 12 h. Serum EPO levels were measured within 6 h and 7 days after the onset of AMI. Ten healthy subjects were controls.

Results
Serum EPO was higher among patients compared to controls. This increase was not related to haemoglobin and had a negative significant correlation with CK and wall motion score index while a positive significant correlation with ejection fraction. Patients with above median EPO level had lower CK and higher ejection fraction compared to those with below median EPO. Serum EPO significantly increased 7 days after the onset of MI and showed significant positive correlation with EPO within 6 h of the onset of MI.

Conclusion
High endogenous EPO level can predict a smaller infarct size in Egyptian patients with acute MI.

Key words: Erythropoietin, acute myocardial infarction, Egyptian Abbreviations: AMI: Acute myocardial infarction, EPO: Erythropoietin, PCI: Percutaneous coronary intervention, CK: creatine phosphokinase

The effect of glucose on hypothalamic neuropeptide Y release investigated using static incubation of hypothalamic explants
Syed Suryan Hussain, Errol Richardson, Niki Buckley, Gavin Bewick, Stephen Bloom & James Gardiner
Imperial College London, London, UK.

Attenuated glucoprivic feeding responses are a feature of hypoglycaemic unawareness in insulin-treated diabetes. Glucose alters the activity of hypothalamic neuropeptides involved in regulating appetite. Accurate nucleus (ARC) Neuropeptide Y (NPY) releasing neurons stimulate feeding. The identification of glucose-sensitive NPY releasing hypothalamic neurones suggests a strong role for these neurones in mediating changes in appetite induced by alterations of glucose. To gain a better understanding of the mechanism involved in glucoprivic feeding, we investigated the changes in NPY release by the hypothalamicas at different concentrations of glucose, using static incubation of hypothalamic explants. Hypothalami from 20 male Wistar rats (mean weight 280 ± 2.3 g) were incubated in artificial CSF (aCSF) for 2 h equilibration period. The hypothalamic explants were then incubated for these 45 min periods in 600 μl aCSF containing 3, 8 (baseline) and 15 mM glucose in randomised order. Finally, the viability of the tissue was verified by 45-min incubation in aCSF containing 56 mM KCl. At the end of each incubation period, supernatants were removed and assayed for NPY release by radioimmunoassay. Only explants that showed a greater secretion of NPY with 56 mM KCl as compared to baseline were considered viable. NPY release in aCSF containing 3 mM, 15 mM and KCl was 285.2 ± 92.8, 130.7 ± 25.8 and 818.8 ± 210.7 percent of basal NPY release, respectively. There was a significant increase in NPY release with aCSF containing 3 mM glucose versus baseline glucose. This supports the presence of glucose-sensitive NPY releasing hypothalamic neurones. These findings are consistent with previous findings that suggest an important role for NPY releasing hypothalamic neurones in stimulating glucoprivic feeding. Defects in the responses of these neurones to glucose may contribute to the development of hypoglycaemia unawareness in insulin-treated diabetes. Our work also supports the use of static incubation of hypothalamic explants to study the effects of glucose on neuropeptides involved in regulating appetite.

Endocrine Abstracts (2011) Vol 25
P157
Study of serum prolactin and cardiovascular risk in patients with type 2 diabetes mellitus
Hisham Elgayar, Manul Abu-Shady, Imran Zaki, Mona Abdelsalam & Alyaa Elsherbeny
Ain Shams University, Cairo, Egypt.

Background
Prolactin is an identified marker associated with atherosclerosis. Atherosclerotic process and hence the macrovascular complications are causes for high mortality and morbidity rates among people with diabetes.

Aim
To assess the relationship between serum prolactin and cardiovascular risk in patients with AMI and patients with type 2 diabetes mellitus.

Subjects and methods
A case-control study was done in Ain Shams University Hospitals, Cairo, Egypt; serum prolactin was determined in 20 non-diabetic (group 1) and 20 diabetic (group 3) male patients within 24 h of the onset of AMI and after 2 weeks. Twenty type 2 diabetic male patients without AMI and 15 healthy age and sex-matched controls represented (group 2 and group 4, respectively). The inflammatory marker hs-CRP was also measured in the studied population.

Results
Serum prolactin was significantly higher among non-diabetic (27.52 ± 6.75 ng/ml) and diabetic patients with AMI (21.05 ± 6.93 ng/ml) compared to the control group (11.3 ± 2.7575 ng/ml, P < 0.01) while the diabetic patients without AMI showed insignificant difference (12.2 ± 3.15 ng/ml) with the control group (P = 1). Group 1 showed minimal decline in serum prolactin level 2 weeks after the onset of AMI (22.56 ± 4.26 ng/ml) but still was significantly higher than the control group (P < 0.01) while group 3 showed marked decline (9.15 ± 2.89 ng/ml) and showed insignificant difference with the control group (P = 0.67). Serum prolactin showed significant positive correlation with hs-CRP among patients with AMI whether diabetic (r = 0.679, P = 0.001) or non diabetic (r = 0.593, P = 0.006).

Conclusion
Hyperprolactinemia may be associated with increased risk of myocardial infarction but the levels of prolactin are variable among diabetic. In addition, the increase in both prolactin and hs-CRP increases the atherosclerotic process and hence the macrovascular complications revealing a new mechanism for atherosclerosis in diabetics.

Key words: Prolactin, acute myocardial infarction, atherosclerosis, diabetes.

Abbreviations: AMI: acute myocardial infarction.

P158
Effect of diet/lifestyle advice on weight change in an unselected polycystic ovary syndrome (PCOS) population
Khusbu Sinha1, Abdullah Albyatti1, Davinia White2, Stephen Franks1 & Lisa Webber2
1Imperial College London, London, UK; 2Imperial College Healthcare NHS Trust, London, UK.

Objectives
To examine the effect on weight change of diet/lifestyle advice to maintain/achieve a healthy weight in an unselected PCOS population.

Methods
All overweight patients attending the Reproductive Endocrine clinic with PCOS are routinely given lifestyle advice for weight loss by their consultant, offered referral to see a dietitian and, when clinically appropriate, prescribed orlistat. Clinical notes were reviewed for 50 consecutive patients with PCOS attending the clinic. Their height, weight and BMI on their first two or three attendances were recorded. Mean weight change between visits was calculated and analysed with reference to starting BMI. Time between the first 2 appointments varied from 1 to 24 months, and 3 to 50 months between the first and third appointments.

Results
Mean weight change for the total group of 50 women between their 1st and 2nd appointments was a gain of 0.1 kg (range –8.6 to +13.7 kg). Twenty-five women gained a mean of 2.3 kg, 20 lost a mean of 2.6 kg and 5 remained unchanged. Thirty-seven women were seen 3 times and their mean weight change between visits 1 and 3 was the same as for the total group (a gain of 0.1 kg; range –18.5 to +13.2 kg). Nineteen of these women gained a mean of 4.4 kg and 18 women lost 4.4 kg. This pattern of weight change was the same for those with a BMI of between 19 and 29.9 kg/m² and for those 30 kg/m² and over.

Conclusions
The overall weight gain of this group over 2–3 visits was very little and less than expected. This suggests that giving lifestyle advice to women with PCOS is at least partially effective.

P159
Investigating the effects of testosterone on inflammatory markers of early aortic atherosclerosis in the testicular feminised mouse (Tfm) model
Daniel Kelly1, Donna Sellers1, Nicola Woodrooffe1, Hugh Jones2 & Kevin Chaner1
1Biomedical Research Centre, Sheffield Hallam University, Sheffield, UK; 2Centre for Diabetics and Endocrinology, Barnsley Hospital NHS Foundation Trust, Barnsley, UK.

Objectives
The over-recruitment and activation of leucocytes characteristic of early atherosclerosis is considered the driving force behind atheroma development and is regulated by the concerted activities of several cytokines, chemokines and adhesion molecules surrounding a lipid core. Low serum testosterone levels are associated with cardiovascular disease in men and clinical trials demonstrate that testosterone replacement therapy (TRT) improves symptoms and reduces the inflammatory burden of atherosclerosis. This study investigates whether the known atheroprotective effect of testosterone in the testicular feminised (Tfm) mouse model1 is associated with an effect on inflammatory factors.

Methods
Tfm mice express a non-functional androgen receptor (AR) and low levels of circulating testosterone. Tfm mice were fed a high-cholesterol diet (42% butterfat, 1.25% cholesterol and 0.5% cholate) ad libitum for 28 weeks and received either physiological testosterone replacement (intramuscular mixed testosterone esters, Sustanon 1002, 25 mg/kg) or placebo (saline) and were compared to wild-type littermate controls. Aortic root serial sections were analysed by oil red O staining and percentage lipid deposition calculated. Presence of inflammatory cells was investigated by immunohistochemistry in addition to expression of the novel inflammatory chemokine, CX3CL1. Blood was analysed for testosterone, 17β-estradiol, lipids and cytokines (TNFα, IL6, IL1β, IL10 and MCP-1). Investigators were blinded to treatment group throughout sample analysis (ANOVA).

Results
TRT reduced lipid deposition in Tfm mice receiving a high cholesterol diet compared to placebo (2.15 ± 0.17 vs 4.70 ± 1.04%, respectively; P = 0.05), but demonstrated no effect on serum lipids and cytokines. Immunohistochemistry detected monocyte infiltration locally adjacent to lipid streaks. CX3CL1 and its receptor were detected in plaque regions but were not influenced by testosterone or AR function (n = 6).

Discussion
Physiological concentrations of testosterone can inhibit fatty streak formation, via AR-independent mechanisms in Tfm mice, although not through systemic anti-inflammatory actions or local effects on chemokine expression.

P160
Study of the adipokine visfatin in obesity and type 2 diabetes mellitus
Salah Shellehaya, Nehad Shoeib, Salwa Seddik, Khalid Makboul, Rania Abd El Baki, Eman Fahmy & Eman El-ghohary
Ain Shams University, Cairo, Egypt.

Background
Visceral fat-derived protein named visfatin was discovered in 2005. Visfatin may improve glucose tolerance, and its plasma levels correlate strongly with the amount of visceral adipose tissue in humans. Visfatin has insulin-like metabolic effects on glucose metabolism but has a distinct binding site on insulin receptors. These findings triggered great interest for many researchers to explore the mechanism(s) of regulation of visfatin expression, and the possible relationships between visfatin and obesity, insulin resistance, and T2DM. However, the available information on visfatin is still too little and controversial.

Aim
The aim is to study the levels of visfatin and its relationship to each of obesity, insulin resistance and T2DM. However, the available information on visfatin is still too little and controversial.

Methods
The study was conducted on 80 subjects divided into Group I a: 20 obese T2DM patients, Group I b: 20 lean T2DM patients, Group II a: 20 obese nondiabetic, Group II b: 20 lean nondiabetic subjects. They were subjected to, full clinical history, thorough clinical examination, fasting plasma glucose (FPG), glycated hemoglobin (HbA1c), fasting plasma insulin (FI), homeostasis model assessment (HOMA-IR), cholesterol, TG, LDL-c, HDL-c, serum visfatin and CT abdomen to assess the visceral (VAT) and subcutaneous (SAT) adipose tissue of the patients chosen randomly.

Endocrine Abstracts (2011) Vol 25
Results
Serum visfatin was found to be higher in T2DM patients than control group as well as in T2DM obese patients than obese control group and in T2DM lean patients than lean control group. Also serum visfatin was higher in obese T2DM patients than T2DM lean patients. There was a highly significant positive correlation ($P \leq 0.001$) between serum visfatin and BMI, FPG, HbA1c, TG, cholesterol, HOMA IR and FI and a significant positive correlation ($P \leq 0.05$) between visfatin and waist circumference and VAT.

Conclusion
These results may postulate the presence of a link between visfatin and diabetes mellitus independent of obesity.

P161
Study of lipoprotein phospholipase A$_2$ as a biomarker for cardiovascular events in type 2 diabetes mellitus
Mohamed El-Gayar, Inas Sabry, Rania Abdl El Baki, Magdy Abass & Abd-El-Azeem Attia
Ain Shams University, Cairo, Egypt.

Background
Type 2 diabetes (T2DM) represents an independent risk factor for cardiovascular diseases (CVD), being characterized by a continuous low-grade inflammation and endothelial activation state. Lipoprotein-associated phospholipase A$_2$ (Lp-PLA$_2$) may have a pro-inflammatory role as growing evidence suggests that it acts in several pathways that contribute to atherogenesis.

Objective
The objective is to study Lp-PLA$_2$ as a possible biomarker for cardiovascular disease in T2DM.

Subjects and methods
A case control study included 45 diabetic subjects who were divided into 3 groups. Group (1): 15 T2DM patients with recent ischemic stroke ($< 24$ h). Group (2): 20 T2DM patients with acute myocardial infarction. Group (3): 10 recently diagnosed uncomplicated T2DM individuals matching age and sex. All individuals were subjected to full history, thorough clinical examination, serum Lp-PLA$_2$ by enzyme-linked immunosorbent assay (ELISA); serum highly sensitive C-reactive protein (hsCRP) by Nephlometry; lipid profile, fasting (FPG) and postprandial plasma glucose (2hPPG), glycated hemoglobin (HbA1c), Electrocardiography (ECG), Echocardiography and computerized tomography (CT) of brain. Cardiac enzymes were done for patients with acute myocardial infarction.

Results
The study showed that there was significant difference between patients with either cerebrovascular stroke and acute myocardial infarction in comparison with uncomplicated T2DM subjects respectively as regards Lp-PLA$_2$, and hsCRP, being higher in patients with cardiovascular complications ($P < 0.05$). Lp-PLA$_2$ was significantly positively correlated with hsCRP only in patients with cerebrovascular stroke ($r: 0.532$ ($P < 0.05$) but not in patients with acute myocardial infarction ($r: -0.22$ ($P > 0.05$)).

Conclusion
Levels of Lp-PLA$_2$ and hsCRP may be complementary beyond traditional risk factor in identifying middle aged individuals at increased risk for cardiovascular events in type 2 diabetes mellitus.

P162
Serum 25-hydroxyvitamin D$_3$ concentrations and cardiovascular risk among type 2 diabetic patients
Salah Shelbaya, Salwa Sedeek, Rania Abdl El Baki & Nesma Ibrahim
Ain Shams University, Cairo, Egypt.

Background
There is accumulating evidence suggesting that altered vitamin D may play a role in the development of T2DM. A growing body of evidence suggests that low levels of vitamin D may adversely affect the cardiovascular system.

Objective
This is to assess the relation of serum 25 OH vitamin D concentrations and risk of CVD in T2DM patients.

Subjects and methods
Seventy subjects aged from 40 to 60 years were chosen excluding those with any other chronic illness and those receiving any medication that affects vitamin D metabolism or taking vitamin D supplement. They were divided into: Group I: 20 subjects with T2DM with CVD, Group II: 20 subjects with T2DM without CVD, Group III: 20 non diabetic subjects with CVD, Group IV: 10 healthy subjects.

Results
Serum 25 OH vitamin D was lower in T2DM patients with CVD than those without CVD than non diabetic patients with CVD than control subjects ($P < 0.01$). hs-CRP level was significantly higher in T2DM patients with CVD and non diabetic patients with CVD than in T2DM patients without CVD ($P < 0.01$). Microalbuminurea was higher in T2DM patients with CVD than those without CVD and non diabetic patients with CVD ($P < 0.01$). There was significant negative correlation between serum 25 OH vitamin D and BMI, waist circumference, systolic blood pressure, diastolic blood pressure, FPG, 2hPPG, HbA1c, TC, TG, LDL-c, hs-CRP and microalbuminurea ($P < 0.01$).

Conclusion
T2DM patients and patients with CVD had a significant reduction in serum 25 OH vitamin D concentrations, so ongoing evaluation of the protective role of vitamin D$_3$ supplementation in the development of atherosclerosis is needed.

P163
Low testosterone predicts increased mortality and testosterone replacement therapy improves survival in men with type 2 diabetes
Vakkat Muraleedharan1, Hazel Marsh1 & Hugh Jones2,3
1Barnsley Hospital NHS Foundation Trust, Barnley, UK; 2University of Sheffield, Sheffield, UK.

Background
Low testosterone in men is associated with increase in all-cause and cardiovascular mortality. There is a high prevalence of hypogonadism in men with type 2 diabetes and testosterone replacement therapy (TRT) improves cardiovascular risk. However there is no published data regarding mortality in these patients in relation to testosterone levels, and the long term effect of TRT on mortality.

Aim
We report a 6 year follow-up study examining the effect of baseline testosterone and TRT in hypogonadal men with type 2 diabetes on all-cause mortality.

Methods
Five hundred eighty-seven patients with type 2 diabetes had total testosterone (TT) performed between 2002 and 2005 and were followed up for 5.8 ± 1.3 years. Deaths during the first 6 months were excluded. Patients were then analysed in three groups. i) normal TT (>10.4 nmol/l) ii) low TT (≤10.4 nmol/l) without TRT, iii) low TT receiving TRT for 2 years or more.

Results
Of 580 patients analysed, 338 had normal TT (58%) and 240 low TT (42%). In the low TT group 58 patients received TRT. Mean age 61 ± 11 s.d. and similarly matched in all three groups. Total deaths 72 (12.4%). Mortality rates – low TT without treatment (36/182:20%), normal TT (31/338:9%) and low TT with TRT (5/58:8.6%). Survival was significantly decreased in patients with low TT without TRT ($P = 0.001$ log rank) compared to normal. The treated group had improved survival ($P = 0.049$ log rank). In the Cox Regression model multi-variate (age, weight, HbA1c, pre existing cardiovascular disease, smoking, statin and ACEi/ARB use) adjusted hazard ratio for all-cause mortality was 2.2 (95% CI 1.3–3.7 $P = 0.001$) for low TT.

Conclusions
This study shows that men with type 2 diabetes and low testosterone have a significant increased mortality. TRT improved survival compared to those untreated, recording a similar mortality rate to the normal TT group.

P164
Chronic GH excess is associated with adenosine monophosphate-activated protein kinase (AMPK) threonine-172 phosphorylation changes that do not lead to changes in AMPK activity
Julia Thomas1, Edward List1, John Kopchick2, Ashley Grossman1 & Marta Korbonits1
1Barts and the London School of Medicine and Dentistry, QMUL, London, UK; 2Ohio University, Athens, Ohio, USA.

GH influences multiple metabolic pathways. Excess GH (acromegaly) causes a distinct form of cardiomyopathy, which may progress to fulminant heart failure. AMPK is an energy conservation enzyme that modulates multiple areas of the cell stress response, inhibiting anabolism and promoting catabolism. AMPK is
activated by phosphorylation at Thr172 and measurement of Thr172 phosphorylation is thought to correlate with enzyme activity. We investigated the influence of GH on cardiac AMPK in transgenic mouse models of chronic GH excess.

Methods
Bovine-GH-overexpressing (bGH) transgenic mice were produced by the microinjection of bGH cDNA into the pronucleus of C57BL/6J embryos. Animals were sacrificed at 2 and 8 months of age. Cardiac tissue was homogenised and AMPK immunoprecipitated using AMPK-α1 and -α2 antibodies. Functional AMPK assay was performed using the synthetic tandem peptide SAM5 and 3P-ATP. Western blotting was performed for total AMPK (tAMPK) and Thr172-phospho-AMPK (pAMPK) using monoclonal antibodies. Bands were corrected for the house-keeping protein GAPDH.

Results
There were no significant differences in cardiac AMPK activity, measured by functional assay, between transgenic mice and their appropriate littermates at either timepoint. However, 2-month-old bGH mice demonstrated an increase in pAMPK/tAMPK ratio compared to controls and 8-month-old bGH mice demonstrated a reduction in pAMPK/tAMPK ratio compared to controls.

Conclusion
Chronic GH excess is associated with alterations in cardiac AMPK Thr172 phosphorylation which do not appear to influence its activity. A discrepancy between AMPK Thr172 phosphorylation levels and the functional assay is unusual and may represent other factors influencing AMPK function, such as phosphorylation at inhibitory sites. The low levels of pAMPK/tAMPK in older bGH transgenics may be a contributing factor to the heart failure of acromegalic cardiomyopathy.

P165
Differential effects of olanzapine and risperidone on plasma adiponectin levels over time: results from a 3-month prospective open-label study
Adrian Heald1, Linda Hanssens2, Ruud van Winkel3,4, Julien Collette2, Joseph Feusken2, Jean Yves Reginster2, Andre Scheen3, Martien Wampers4 & Marc de Hert4
1Leighton Hospital, Crewe, UK; 2University of Liege, Liege, Belgium; 3Maastricht University Medical Centre, Maastricht, The Netherlands; 4University Psychiatric Centre Catholic University, Leuven, Kortenberg, Belgium.

Aims
Second-generation antipsychotics (SGA), especially clozapine and olanzapine, are associated with increased metabolic risk. Plasma adiponectin levels, vary in schizophrenia patients as in the general population according to gender, adiposity and metabolic syndrome (MetS).

Here, we investigated whether different SGAs differentially influence plasma adiponectin levels independent of body mass index (BMI) and MetS status.

Methods
One hundred and thirteen schizophrenia patients (65.5% males) who were free of antipsychotic medication were enrolled in this open-label prospective single-centre study and received either risperidone (n = 54) or olanzapine (n = 59). They were followed-up for 12 weeks. Average daily dose was 4.35 mg/day for risperidone and 17.36 mg/day for olanzapine. Plasma adiponectin levels as well as fasting metabolic parameters were measured at baseline, 6 and 12 weeks.

Results
Baseline BMI (23.7 ± 23.2 kg/m²), mean fasting plasma glucose (FPG) (mean fasting plasma insulin (11.3 ± 12.0 mU/ml) and mean plasma adiponectin levels (10 154 ± 11 280 ng/ml) were similar in the risperidone group and in the olanzapine group. A significant increase in body weight occurred over time in the olanzapine group as opposed to a numerical increase in the risperidone group (+7.0 kg vs +3.1 kg, P < 0.05). Changes in MetS prevalence, in glucose and insulin levels and in HOMA-IR were not significant.

Conclusion
We observed a significant (P = 0.001) treatment by time interaction showing an adiponectin increase in the risperidone-treated patients (from 10 154 ± 11 280 ng/ml) but an adiponectin decrease in olanzapine-treated patients (from 11 280 ± 8988 ng/ml). This effect was independent of BMI and the presence/absence of MetS.

P166
Lack of beneficial metabolic profile in liver-specific 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) knockout mice
Gareth Lavery, Elizabeth Rabbitt, Agnieszka Zielinszka, Beverly Huges, Nina Semjonous, Khalid Saqui, Stuart Morgan, Laura Gathercole, Elizabeth Walker & Paul Stewart
University of Birmingham, Birmingham, UK.

In humans glucocorticoid (GC) excess can promote hepatic glucose and triglyceride production contributing to obesity, fatty liver and diabetes. 11β-HSD1 converts GCs (11-DHC to corticosterone in mice), thereby increasing tissue concentrations. The liver has the highest 11β-HSD1 activity, and its inhibition has emerged as a therapeutic option. To investigate this we generated 11β-HSD1 liver-specific knockouts (HSD1LKO) and examined GC metabolism and responses to high-fat diet (HFD).

We generated HSD1LKOs using an albumin-Cre transgenic mouse and showed abolished 11β-HSD1 activity only in the liver; 11β-HSD1 knockout (HSD1KO) mice were used as additional controls.

An oral cortisone challenge resulted in serum cortisol concentrations of 1830 ± 184 nm (control), 691 ± 70 nm (HSD1LKO) and 67 ± 24 nm (HSD1KO) (control vs. HSD1LKO P < 0.002, n = 7–9), indicating significant extra-hepatic GC production in HSD1LKOs. Corticosterone/11-DHC urinary metabolite ratios were the same in HSD1LKO and controls (0.08) and elevated (0.5) in HSD1KOs, indicating that despite diminished hepatic GC production there is no impact on hepatic metabolism set-point in HSD1LKOs. HSD1LKO and controls had similar basal serum corticosterone concentrations and adrenals were only significantly larger in HSD1KOs. HSD1LKO and controls were alike for body, liver, epididymal fat pad and muscle weight. No differences were observed for fed and fasting glucose and insulin levels on regular and HFD. HFD induced glucose intolerance to similar levels in both HSD1LKO and controls subjected to glucose tolerance tests. Both HSD1LKO and controls developed fatty liver on HFD assessed by Oil Red O staining and hepatic triglyceride (TAG) assays, and no differences were observed between serum TAG, NEFA and cholesterol profiles.

Despite the loss of hepatic GC regeneration, HSD1KOs had no protection from the metabolic consequences of a HFD as seen in HSD1KOs. This model produces a novel insight into the target for 11β-HSD1 inhibition in terms of improving metabolic homeostasis and suggests a primary role for extra-hepatic enzyme expression.
human development identified multiple loci of interest. For instance, compared to our data from adult islets (Gaulton et al. Nature Genetics 42 255–259, 2010) INSULIN was largely silenced in progenitor cells whereas the adjacent IGF2 locus was active in progenitors but silenced in adult islets. Taken together, these data define for the first time when multipotent human pancreatic epithelial cells are present facilitating opportunities to determine what regulates their differentiation into different pancreatic cell lineages. Discovering these data will underpin either beta-cell regenerative approaches or the assessment of insulin-secreting cells generated from human ES cells.

**Endocrine tumours and neoplasia**

**P168 Ectopic ACTH syndrome: experience of a tertiary referral centre: from diagnosis to outcome**

Andrea Velozla, Georgia Ntali, John Wass & Nikki Karavitaki
Department of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK.

**Introduction**
Ectopic Cushing’s disease (ECS) accounts for approximately 10% cases of Cushing’s syndrome. Its recognition may be delayed and its diagnosis and treatment remain challenging.

**Aim**
To analyze the clinical, biochemical, radiological features, as well as the outcome of patients with ECS presenting in a tertiary referral centre.

**Material and methods**
The records of patients with ECS followed presenting in our Department between 1/1996–1/2010 were reviewed.

**Results**
Thirteen patients were identified (7 men). The mean (±s.i.s) age of onset of symptoms was 42.7±13.8 years (range 13–67). The period between onset of symptoms and diagnosis of hypercortisolism was 10.5±8 months (1–24). The most frequent reported manifestations were muscle weakness (85%), weight gain (85%), easy bruising (69%), hypertension (62%), hypokalemia (62%). 100% (8/8) of the patients did not respond to CRH test and 89% (8/9) did not suppress on the high dose Dexamethazone suppression test. No subject (n = 5) showed gradient on the bilateral inferior petrosal sinus sampling. The localization of the source of the ECS was achieved between 1 and 90 months; in 9 it was of lung origin (8 pulmonary carcinoid, 1 small cell lung cancer), 1 had gastric adenocarcinoma, 1 prostate adenocarcinoma, 1 intestinal carcinoid tumour and 1 occult tumour. The median duration of follow-up was 27 months (1–148). 39% (5/13) of the subjects died within a median period of 6 months (1 pulmonary carcinoid, 1 small cell lung cancer, 1 gastric adenocarcinoma, 1 prostate adenocarcinoma, 1 occult tumour). Amongst the 8 patients with pulmonary carcinoid, tumour resection was performed in 7 (with lymph node removal/biopsy in 5 and proven metastatic tumour). Amongst the 8 patients with pulmonary carcinoid, tumour resection was performed in 7 (with lymph node removal/biopsy in 5 and proven metastatic disease in 2); no evidence of recurrence was documented in 71% (5/7).

**Conclusions**
ECS is a heterogeneous disease mainly attributed to lung origin. Tumour localization may be delayed necessitating regular imaging. Mortality primarily attributed to the original tumour is significant.

**P169 Ovarian steroid cell tumour in association with Von Hippel–Lindau disease**

Jenny Prouten, Sana Mota & Malcolm Littley
East Lancashire Hospital Trust, Blackburn, UK.

**Case**
A 46-year-old woman presented to the gynecology department with secondary amenorrhoea, hirsutism and acne. At the age of 25, she was diagnosed with Von Hippel–Lindau disease (VHLD) on the basis of multiple right renal haemangioblastomas. Investigation revealed an elevated testosterone 13.4 nmol/l (0.3–2.6 nmol/l), 17 hydroxyprogesterone 18 nmol/l and androgen index 53.6 (0.0–7.5). A solid lesion in the left ovary was demonstrated on ultrasound scan of the pelvis. This was confirmed by computerised tomography (CT), where a right adrenal mass measuring 21.4 x 22 mm in dimension and a cortical cyst in the lower pole of the left kidney were incidentally noted. An endocrinology opinion was sought prior to exploratory surgery.

Twenty-four-hour urinary metanephrine and normetanephrine levels plus serum dihydroepiandrosterone sulphate (DHEAS) were within normal limits. Biopsy of the left ovary revealed a steroid cell tumour strongly positive for calretinin. She underwent total abdominal hysterectomy and bilateral salpingo-oophorectomy. Post operatively testosterone levels normalised and the features of virilisation regressed.

**Discussion**
Von Hippel-Lindau disease is an autosomal dominant condition with an incidence of 1 in 36 000 live births. It is characterised by CNS haemangio-blastomas and visceral tumours. The VHL gene is a tumour suppressor gene found on chromosome 3. Gonadal involvement is in the form of epididymal cystadenomas in men and broad ligament cystadenomas in women. Steroid cell tumours are rare, accounting for <0.1% of ovarian tumours. They are unilateral in 96% of cases, and 30% are malignant. They commonly secrete androgens and present with virilisation, often in the 3rd or 4th decade. The management is surgical.

**Conclusion**
We report an unusual case of an ovarian steroid cell tumour in association with VHLD. This highlights the need to remain vigilant for unusual pathologies and a possible link between the two diseases.

**P170 Simplified minimally invasive parathyroidectomy: a series of 100 cases and a review of the literature**

William Wong, Fung Jun Foo, Michael Lau, Ashima Sarin & Pasupathy Kiruparan
Blackpool Victoria Hospital, Blackpool, Lancashire, UK.

**Background**
Minimally invasive parathyroidectomy (MIP) is usually performed with the concurrent use of intraoperative adjuncts for good outcome.

**Objective**
We wanted to show that a good success rate can be achieved in MIP without routine use of any intraoperative adjuncts.

**Methods**
A prospective case series of the first 100 patients who underwent MIP for primary hyperparathyroidism by a single surgeon at a single institution were included in this study. Preoperatively, patients undergo ultrasonography and/or 99 mTc-labelled sestamibi scan for localization. Methylene blue contrast is used preoperatively. Parathyroidectomy is performed via a focussed lateral approach on the side indicated by preoperative imaging. An algorithm of intraoperative decisions is followed. No intraoperative adjuncts such as gamma probe, intraoperative PTH or frozen sections are used routinely. Patients are followed up in the outpatient clinic, where serum calcium and/or parathyroid hormone levels are checked to determine success. Postoperative normocalcaemia is considered success independent of serum PTH levels.

**Results**
Patients had a median age of 63 years (range 26–85 years). Eighty-three were female and 17 were male. Ninety-three patients underwent MIP, with 7 patients having a conversion from MIP to bilateral exploration. The mean operative time for unilateral and bilateral exploration was 42.38 ±12.31 and 76.43 ±16.51 min respectively. When used separately MIBI and USS were able to accurately lateralise side of the lesion in 82.8 and 79.5% respectively but when USS and MIBI agreed, the predictive accuracy of the side of the lesion was 87.5%. Ninety-six percent of patients had a successful return to normocalcaemia. No intraoperative or postoperative complications were encountered.

**Conclusion**
Excellent results are achievable with MIP even without intraoperative adjuncts. Preoperative localisation is helpful in determining side of incision. Our technique demonstrates a key principle of surgery: to keep things simple.

**P171 ‘Uterine neuroendocrine tumour: an unusual cause of hyponatraemia’ and the role of tolvaptan, a vasopressin V2 receptor antagonist**

Gideon Mlawa, Laura Fraser, Sophie Price, Robert Green, Ben Turner & Rowland Guy
Basingstoke and North Hampshire Foundation Trust, Basingstoke, UK.

**Background**
Hyponatraemia is the commonest electrolyte abnormality in clinical practice, and may be a biochemical manifestation of different diseases including malignancy.

**Endocrine Abstracts (2011) Vol 25**
Uterine neuroendocrine tumours causing hyponatraemia are rare and can cause a diagnostic challenge. We present a case of 68 years widow who presented with 3 weeks history of nausea, occasional vomition, confusion, increased urinary frequency, urinary incontinence, chronic constipation, and weight loss. Her past medical history included left knee arthroscopy, cocktail implant and sterilisation in 1976. On admission she was haemodynamically stable with normal observations. Blood test revealed profound hyponatraemia (Na+ 118 mmol/L), and serum osmolality of 247 mOsm/kg, urine osmolality of 701 mOsm/kg, urinary sodium of 110 mmol/L, random cortisol of 542 mmol/L, in keeping with SIADH. Tumour markers were negative. Staging CT revealed heterogeneous enhancing uterine mass. Flexible sigmoidoscopy was normal. Her medications included Paracetamol, Codeine Phosphate and laxatives. She was started on fluid restriction of 750 ml/24 h. She remained hyponatraemic (116–117 mmol/L) despite this. Oral Demeclocycline 300 mg TDS was added, but she was unable to tolerate it and was started on tolvaptan (30 mg od). Discharged on tolvaptan (30 mg) and was reviewed by gynaecology team in outpatient clinic. Cervical biopsy histology showed a high grade neuroendocrine malignant tumour confirmed by positive immuno labelling for chromogranin, AE1/3 and CAM5.2. She is currently undergoing chemotherapy and still on tolvaptan. Her sodium remains low but at safer levels of 127–130 mmol/L.

Discussion
Hyponatraemia secondary to uterine neuroendocrine tumours is rare and generally associated with chemotherapy treatment rather than being related to the uterine neuroendocrine tumour. Conventional treatment for hyponatraemia due to SIADH was ineffective or not tolerated. Tolvaptan treatment was more successful even though it is mainly used in patient with mild to moderate euvaloaemic hyponatraemia. This case report demonstrates the possibility of using tolvaptan in severe hyponatraemia.
P175
Phaeochromocytoma presenting as polycythaemia
Ian Seetho & Koshy Jacob
Department of Diabetes and Endocrinology, United Lincolnshire Hospitals NHS Trust, Pilgrim Hospital, Sibsey Road, Boston, Lincolnshire, UK.

Case
A 35-year-old man was referred by the haematologists. He had been previously diagnosed with dilated cardiomyopathy and later underwent a cardiac transplant. Following this, he suffered an anterior wall myocardial infarction with subsequent congestive cardiac failure. A coronary angiogram did not reveal any evidence of allograft coronary artery disease. His medications included diuretics, immune-suppressants and warfarin. There was no significant family history. Clinical examination revealed a pansystolic murmur but was otherwise unremarkable. He was found to have polycythaemia and he was investigated for this by the haematologists. His CT scan abdomen revealed a left supra renal mass and the biopsy histology was consistent with an adrenal phaeochromocytoma.

The 24 h urinary catecholamines excretion was consistent with the diagnosis. (Nonmetadrenaline excretion 21 446 nmol/d (0–490); Metadrenaline 5578 nmol/d (0–430); Metadrenaline 529 nmol/d (0–2000); Adrenaline excretion 29 nmol/d (0–70); Dopamine excretion 927 nmol/d (0–2700)). His Methyl-iodo benzyl guanidine (MIBG) scan showed focal increased uptake of the left adrenal. An MRI of the CNS did not yield features to suggest Von Hippel Lindau and genetic tests did not show pathogenic mutations of VHL, RET, SDHB, SDHC and SDHD genes.

Discussion
This man was diagnosed with phaeochromocytoma that was found during investigations for polycythaemia. Although an elevated haematocrit has been observed in association with phaeochromocytomas, the occurrence of absolute polycythaemia in such cases is not common. The pathogenesis may involve induced unregulated erythropoietin secretion from the tumour, resulting in secondary erythrocytosis. Awareness of this association is important in order that either diagnosis is not delayed and definitive management can be established. Reversal of alpha-mediated vasoconstriction may lead to haemodilution and curative surgical resection of the phaeochromocytoma can result in reduction in erythropoietin levels with regression of the polycythaemia.

P176
Development and validation of a LC-MS/MS method for the measurement of plasma renin activity using on-line solid phase extraction
Stephanie Carter, Laura Owen & Brian Keevil
University Hospital of South Manchester, Manchester, UK.

The measurement of plasma renin activity is required in a number of clinical situations, in particular screening for primary aldosteronism (PA) and monitoring mineralocorticoid replacement therapy. PA is a treatable cause of hypertension and has an estimated prevalence of up to 20% amongst resistant hypertensives. Consequently, recent guidelines now recommend screening for PA in all patients groups with a high prevalence of PA. At present, the most reliable method of screening for PA is to calculate the plasma aldosterone to renin ratio (ARR), with a raised ARR being suggestive of PA.

The plasma renin activity (PRA) assay measures the ability of plasma renin to generate angiotensin I (Ang1) from endogenous angiotensinogen, with all established assays using an immunnoassay to quantitate AngI. We have now developed and validated a method for the measurement of PRA which involves extraction and quantitation of AngI by online solid phase extraction-LC-MS/MS. This method requires a sample volume of 50 μl and has an intra-assay precision <7% across the working range of the assay. A 6.5 h incubation step gave a LLOQ of 0.3 nmol/l per hour and this can be reduced to 80 pmol/l per hour using a 24 h incubation, allowing the measurement of very low activity samples. In comparison to the established immunoassays, this method will potentially improve assay specificity and will also reduce turnaround time, consumable costs, staff time, sample handling and sample volume requirement.

There is uncertainty in the literature regarding the stability of renin at room temperature and also the extent to which prorenin cysactivation occurs. We have now studied the stability of PRA, with preliminary results suggesting that PRA is unaffacted by storage of whole blood at room temperature for at least 24 h. This would enable us to analyse blood samples taken in primary care, potentially providing more effective screening of the hypertensive population.

P177
Clinical and biochemical features of sporadic and hereditary phaeochromocytomas and paragangliomas: an analysis of 47 cases investigated in a single centre
Shahina Begum, Paul Carroll & Barbara McGowan
King’s College London, London, UK.

Introduction
Advances in the understanding of the natural history and genetics of phaeochromocytomas and paragangliomas have altered the demographics of these conditions resulting in much higher rates of malignancy and association with known genetic abnormalities.

Objective
To analyse the clinical and biochemical features of hereditary (H) and sporadic (S) phaeochromocytomas and paragangliomas.

Design
Retrospective case-series at Guys and St Thomas’ NHS Foundation Trust identified using our in-house electronic database (Diabeta-3).

Results
Forty-seven patients were reviewed over a period of 30 years, 36% (n=17) of these patients had hereditary phaeochromocytomas or paragangliomas (SDHB 5, SDHD 2, VHL 5, RET 3, NF1 2). The hereditary group presented at a significantly younger mean age (S: 44.7 ± 15.3 years, H: 29.5 ± 16.9 years, P=0.006). The hereditary group had significantly more bilateral adrenal phaeochromocytomas (S: 4.5%, H: 45.5%, P=0.03) and a positive trend for multiple paragangliomas, although not statistically significant.

Sporadic tumours were bigger than hereditary but this was not significant (S: 6.39 ± 1.07 cm, H: 3.43 ± 0.85 cm, P=0.25). All patients showed the classic triad of headaches, palpitations and diaphoresis as the most commonly reported symptoms, but hypertension was more prevalent in the sporadic group (S: 60%, H: 35.3%). Urine catecholamine concentration was highly variable and failed to show any significant difference between both groups. Adrenal phaeochromocytomas secreted more noradrenaline than paragangliomas regardless of their classification. There was no significant difference between the malignant potential of tumours however only adrenal phaeochromocytomas showed malignant change.

Conclusions
The data suggest that patients with hereditary phaeochromocytomas and paragangliomas present at a younger age with more bilateral tumours compared to the sporadic group. There may be a higher susceptibility for malignancy in adrenal tumours compared to extra-adrenal tumours. It is difficult to differentiate the 2 groups based on clinical presentation, size and features of catecholamine secretion. Genetic testing remains key to differentiating between sporadic and hereditary disease.

P178
Metastatic insulinoma treated by transhepatic arterial embolisation
E Marie Freet1, Claire McDougall1, Karen Campbell1, Donna Grant1, Ram Kasthuri2 & Nicholas Reed1
1Department of Endocrinology, Western Infirmary, Glasgow, UK; 2Department of Radiology, Gartnavel General Hospital, Glasgow, UK; 3Beacon West of Scotland Cancer Centre, Glasgow, UK.

A 64-year-old man (HM) was admitted to our local hospital with transient dysarthria and right hemiparesis; formal blood glucose was 2.2 mmol/l on admission. During his in-patient stay, he had frequent episodes of hypoglycaemia. Further questioning revealed a 4 week history of recurring dizzy spells which improved on eating.

Cerebral imaging and adrenal function were normal and so the patient underwent further investigations (summarised below) which confirmed insulinoma.

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (&lt;13 mU/l)</td>
<td>166</td>
<td>172</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>2.3</td>
<td>2.1</td>
</tr>
<tr>
<td>C-peptide (0.4–1.1 nmol/l)</td>
<td>1.8</td>
<td>1.7</td>
</tr>
<tr>
<td>Insulin–glucose ratio (&lt;5)</td>
<td>276</td>
<td>429</td>
</tr>
</tbody>
</table>

In addition, plasma chromogranin-A was elevated (590 pmol/l) consistent with a neuroendocrine tumour. Abdominal imaging demonstrated a thickened pancreatic tail and widespread liver metastases; these were octreotide avid on
octreoSPECT-CT scanning. Subsequent liver biopsy confirmed metastatic insulinoma. Primary surgical cure was impossible and HM required a combination of diazoxide, octreotide and dexamethasone to control hypoglycaemia. HM subsequently underwent transhepatic arterial embolisation to treat his metastatic liver disease. This has resulted in normalisation of blood glucose on sandostatin LAR and reducing dose dexamethasone.

Insulinomas are rare tumours of the pancreatic islet cells with an incidence of ~4 per million/year; around 5–10% of cases are malignant. When surgical cure is unattainable alternative palliative treatments include medical therapy, radiofrequency ablation or arterial embolisation to hepatic metastases. The response rates associated with embolisation (decreased hormone secretion/symptomatic benefit/radiographic regression) are over 50%. However, the duration of response can be brief, ranging from 4 to 24 months in uncontrolled series.

Everolimus, a rapamycin analogue, is a new oral mTOR inhibitor. In a recent study, everolimus associated with regression of neuroendocrine tumours and there is a benefit/radiographic regression) are over 50%. However, the duration of response can be brief, ranging from 4 to 24 months in uncontrolled series.

We assessed the functionality of adrenal incidentalomas referred to the endocrinology team at the Queen Elizabeth Hospital in Gateshead.

Methods

Patients with adrenal incidentalomas that were referred to the endocrinology team between January 2008 and August 2009 were identified. Patient notes, laboratory results, images and radiological reports were reviewed.

Results

Sixty-two patients (27 females) were referred to the endocrinologists with a finding of an incidental adrenal nodule with sizes ranging between 6 and 42 mm. Twenty-nine (47%) patients had left sided tumours, 23 (37%) were on the right and 10 (16%) were bilateral. All patients underwent a series of hormonal investigations: 94% had 24 h cortisol levels measured. 87% had an overnight dexamethasone suppression test, 95% had urinary metadrenalines measured and aldosterone/renin activity was measured in 90% of patients.

The largest group of patients (87%) were found to have benign non-functional adenomas. One patient had a pheochromocytoma, I had a cortisol secreting adrenal mass, and 6 (10%) patients were found to have sub-clinical Cushing’s syndrome. No patients were found to have primary hyperaldosteronism. Four (6%) patients (one with overt Cushing’s syndrome, one with a pheochromocytoma and two with subclinical Cushing’s) underwent definitive surgical management.

Conclusions

Nearly 13% of patients that are referred with adrenal incidentalomas are functional on subsequent endocrine evaluation. Potentially serious conditions such as pheochromocytoma and overt Cushing’s syndrome can be identified and treated. However, the most common functional abnormality is subclinical Cushing’s syndrome for which there is current lack of sufficient evidence regarding its long term management.
A rare case of calcitonin and carcinoembryonic antigen negative medullary thyroid cancer
Anjali Santhakumar, Sebastien Aspinal & David Woods
Wansbeck General Hospital, Northumberland, UK.

Background
Routine measurements of serum calcitonin levels are considered an integral part of the diagnostic evaluation of medullary thyroid cancer (MTC). We report a rare case of calcitonin and carcinoembryonic antigen (CEA) negative MTC.

Case presentation
A 63-year-old retired plasterer attending a well-mens clinic was referred to the endocrinology service with elevated calcitonin (2.75 mmol/dl). Systemic examination was unremarkable and there was no endocrine history of note in the family. Repeat calcitonin at the clinic was 2.76 mmol/l (2.12–2.60) and PTH level was 76 ng/l (10–60). Twenty-four hour urinary calcium was 11.2 mmol/24 h (2.5–7.5). He was investigated with neck ultrasound and bone densitometry for suspected primary hyperparathyroidism. The bone scan showed osteopenia and the ultrasound showed a 2.5×1.7×1.4 cm dominant left thyroid hyperechoic nodule with specks of calcification.

Calcitonin level was <11 ng/l. CEA was normal at 4.1 µg/l (<5). Overnight urinary metanephrine and normetanephrine levels were normal 0.06 µmol/mmol creat (0–0.30) and 0.12 µmol/mmol creat (0–0.35) respectively. Plasma metanephrines and normetanephrines were 180 pmol/l (120–1180) and 267 pmol/l (80–510) respectively.

Fine needle aspiration of the thyroid nodule under ultrasound guidance was reported as THY3 and he was referred to the endocrine surgeon.

He underwent bilateral neck exploration along with left hemithyroidectomy. Post surgery his histology report was consistent with MTC and he went onto completion thyroidecmy. RET proto-oncogene mutation is awaited.

Discussion
To this date reports of calcitonin and CEA negative MTCs are rare in the English literature. This case illustrates that the diagnosis of MTC cannot be excluded by means of a negative serum calcitonin. When serum calcitonin is negative a cytomorphologic analysis of fine needle aspiration and RET proto-oncogene mutations are two diagnostic tools which are available for detection and accurate management of patients in whom MTC is suspected.

Endocrine disorders in adults treated for Hodgkin lymphoma in childhood
Golsa Ehteshamirad, Rachel Johnson, Judith Kingston & Maralyn Druce
Barts and the London School of Medicine, London, UK.

Introduction
Successful treatment for childhood-onset Hodgkin lymphoma (HL) has highlighted long-term effects of therapy. We review endocrinopathy in HL survivors attending a hospital follow-up clinic and consider, together with published data, appropriate disease screening and monitoring.

Method
Retrospective review of notes and investigations from survivors of childhood HL ≥5 years from diagnosis.

Results
Forty-four subjects were included (30 male, 14 female). Mean age at diagnosis was 10.6±3.5 years; mean follow-up 23.6±8.3 years. Thirty-five (79.5%) had received chemotherapy with or without radiotherapy. Thirty-two (72.7%) had received radiotherapy, 29 including the neck (median dose 35 Gy).

Mean height was 163.0 cm (females) and 175.5 cm (males). Median BMI was BMI 27.5 (females) and 24.5 (males) with 27 subjects (61%) of BMI ≥25.

Routine diabetes mellitus screening was not performed but there were no diagnosed cases. Mean total cholesterol (n = 27) was 4.8 mmol/l in females and 5.5 mmol/l in males; 17 subjects had cholesterol ≥5 mmol/l.

Where documented, for females, mean age at menarche was 13.7 years. Three women (all over 40 years) had LH/FSH ≥20 U/l, with a further 4 on HRT or contraceptive pill. In 10 females with reproductive data available; 7 had had spontaneous pregnancies (9 offsporrng).

In males, mean testosterone was 12.9 nmol/l (n = 26) and 2 patients had required testosterone replacement. Seven men with azoospermia and 3 with oligospermia were noted—all had had chemotherapy (one with additional bone radiotherapy). Thyroid status was available for 43 subjects; 11 (25.6%) had no disease, 30 (69.8%) were taking thyroxine (mean dose 124 mg daily). Of these, 26 (60.5%) had nodular disease (25 of whom had received neck radiotherapy), and 2 had had differentiated thyroid carcinoma.

Conclusion
Long-term follow up, identification of risk factors and careful documentation of late effects together with appropriate screening may enable identification and treatment of morbidity in patients cured of childhood HL.

A patient with adrenal carcinoma
Thet Koko, Andrew Pettit & Cornuelle Parker
Airedale General Hospital, Keighley, UK.

Background
We describe a 64-year-old lady with past medical history of Thalassaemia Trait and Hypertension, who was presented with Cushing’s syndrome.

Clinical presentation
This patient was admitted with severe bi-basal pneumonia in February 2010 and treated successfully. But unfortunately her symptoms persisted with increasing facial swelling in end of May 2010, therefore, a staging CT was arranged and found incidental 6×5.5 cm left adrenal mass which was initially thought to be 2 separate left adrenal and renal masses with no evidence of metastasis or other primary site. She also had weight gain, facial plethora, thinning of the skin, easy bruising, moon face and increased subcutaneous fat deposition in the nape of the neck. Her 24 h urinary free Cortisol levels were raised at 552 and 736 (normal 10–147). She had an over night 2 mg Dexamethasone suppression test which showed failure to suppress cortisol at 537 and ACTH was fully suppressed at
<5.0. She also had a plasma renin activity:aldosterone, plasma metanephrine, 24 h urinary metanephrines (2 samples), DHEA, Androstenedione which were all normal. LH, FSH and oestradiol levels were post-menopausal range. She underwent radical left adrenalectomy and left nephrectomy in June 2010 with hydrocortisone cover peri-operatively. Histology showed left adrenocortical carcinoma with penetration into the capsule and adjacent fat. We have started Mitotane 1 g BD along with Hydrocortisone 10/55 postoperatively and referred to Neuroendocrine Oncology team. She has been continuing to complain of significant fatigue and lethargy despite of adequate hydrocortisone supplement. She is due for hydrocortisone day curve and staging CT in a few weeks time.

Conclusion

Our patient is experiencing fatigue and lethargy for a prolonged period of time without a clear reason, after radical surgery, within the therapeutic range of Mitotane (14–20) and adequate hydrocortisone replacement.

P186

Adrenal incidentaloma: how frequently do adrenal incidentalomas cause problems in terms of tumour growth or hormone hypersecretion?

Patricia Richters1,2 & John Wass3


Background

Adrenal incidentalomas are becoming increasingly common due to the improvements in imaging techniques, increasing numbers of radiological investigations and an ageing population. The current follow-up protocols are designed to avoid missing clinically relevant lesions (e.g. malignancy or hormone hypersecretion) by recommending repeated radiological and biochemical investigations. Large amounts of money are spent on patients who as it seems mostly have benign and non-functioning lesions that warrant no further treatment.

Objectives

The aim of this study is to assess how frequently these tumours cause problems in terms of tumour growth, malignancy and/or hypersecretion of hormones.

Methods

The design of the study is a retrospective study based on patient records. Eighty-five patients (39 females and 46 males) were investigated. General data (age, sex, etc), imaging characteristics, hormone work-up and diagnoses were collected and analysed. The number of operations has also been included.

Results

The median age at discovery of the adrenal incidentalomas was 63 years old (range 20–84). The prevalence of NFAs was highest in the age group from 60 to 70 years old. The median size of the adrenal incidentalomas when discovered was 2.3 cm (range 0.7–22 cm). During the follow-up only one mass (1%) showed a significant increase in size, all the other masses remained stable. None of the incidentalomas became functional (excessive hormone production) during the follow-up period. Four (5%) patients were diagnosed with adrenal cortical carcinoma, 56 (69%) with NFA, two (3%) with Cushing’s, 5 (6%) with pheochromocytoma, 1 (1%) with Conn’s and 74% testing of Aldosterone secretion. Radiological reporting of size of lesion also showed no significant increase in the number of lesions that were judged to be malignant.

Discussion

The majority of the incidentalomas were benign and non-functioning. However, a considerate number of functional and malignant tumours was found. Only one mass progressed during the follow-up period. A cost-effectiveness evaluation is necessary to assess how the expenses that are currently spent on the follow-up of benign tumours can be minimised.

P187

Phaeochromocytoma, paraganglioma and tumour genetics: clinical practice lagging theory?

Umasathan Sinnathamby1,2, Anirupa Sivathasan1,3, Romain Akhtar1, Daniel Berney1, Eamonn Maher2 & Shern Chew1

1St Bartholomew’s Hospital, London, UK; 2University of Birmingham, Birmingham, UK.

Background

Up to a third of subjects who develop a phaeochromocytoma or a paraganglioma will do so as the result of mutations in one of several familial genes. Identifying a causative mutation may have significant implications for family screening and future disease surveillance.

Objective

To review the frequency and type of genetic testing undertaken in subjects presenting to our unit over a 20-year period who have developed a phaeochromocytoma and/or paraganglioma.

Results

A retrospective examination of pathology and genetic databases from our unit identified 160 subjects who developed a phaeochromocytoma and/or paraganglioma between 1989 and 2009 (in whom data was available). Of the 160 subjects, 61% of subjects developed a phaeochromocytoma, 42% developed a paraganglioma. Seventy-eight percent of subjects where followed-up by an endocrinologist while 22% where follow-up by other specialties. Eighty-four percent of subjects have been under long term follow-up while the remaining 16% were not followed-up.

Only 35% (56/160) of subjects have undergone genetic testing but 70% of subjects have been appropriately considered for genetic analysis if testing is not determined mandatory for subjects over the age of 30 years. Of those subjects who had genetic analysis, approximately two-thirds of subjects were tested for both VHL and SDH mutations while 10% were tested for RET mutations.

A substantial minority of subjects have not had appropriate genetic assessment. Some were seen prior to the advent of routine genetic testing. Old databases need to be re-examined and genetic testing reconsidered. Future services for these subjects must be integrated between relevant sub-specialties so that genetic analysis may be appropriately considered.

P188

Clinical outcomes of adrenal incidentalomas over a 3 year period: a retrospective analysis to evaluate a new referral pathway

Ioannis Dimitropoulos1, Roanna George1, Emma Pickering2, Lynne Bower2, Mike Waterson1 & Jamie Smith1

1Department of Diabetes and Endocrinology, South Devon Healthcare NHS Foundation Trust, Torquay, Devon, UK; 2Department of Clinical Biochemistry, South Devon Healthcare NHS Foundation Trust, Torquay, Devon, UK.

Aims

Prior to 2007, there was no agreed guideline at our hospital for the management of adrenal incidentalomas and referrals to our endocrine service were rare. Following evidence-based guidelines, we have developed a local protocol for the assessment of adrenal incidentalomas. We now report clinical outcomes of adrenal incidentaloma patients referred to our endocrine service since its implementation.

Methods

Using a retrospective analysis we collected data on 47 patients with adrenal incidentalomas presenting to our endocrine service over 3 years (2007–2010). The patient list was generated by biochemistry records and other clinical data were accessed from medical notes and radiology reports. Audit standards were based on international consensus guidelines (AACE/AAES Guidelines 2009, Young WF. The incidentally discovered adrenal mass NEJM 2007) for the management of adrenal incidentalomas.

Results

With respect to endocrine investigations, of the 47 cases of adrenal incidentalomas identified (age range, 19–86 years, 20 males, 27 females) 89% were referred to Endocrinology. 26% were diabetic and 45% hypertensive. 100% underwent urinary measurement of catecholamines, 60% testing of Cortisol axis and 74% testing of Aldosterone secretion. Radiological reporting of size of lesion was present in 100% whereas density measurement in Hounsfield units in only 28%. Mean size of lesion was 3.4 cm (range 1.1–10 cm). Thirteen (28% of total) of lesions where >4 cm. Adrenalectomy was undertaken in 17%. Hormonally active lesions were identified in 8.7% and included 3 Phaeochromocytomas and 2 sub-clinical Cushing’s.

Conclusions

Adrenal incidentalomas represent an increasingly common clinical problem. The development of our guideline has led to a significant increase in the referral rate of adrenal incidentalomas and enabled a more systematic endocrine evaluation. Amongst referrals, hormonally-active lesions are relatively common and have been amenable to surgical cure. There are however, areas for improvement in order for our service to comply with international standards of best practice.
P189
Pituitary apoplexy: headache and beyond
Mureed Hussain, Gabriel Yacoub & Haris Rathur
Tameside General Hospital, Ashton Under Lyne, UK.

Case 1
A 43-year-old man presented with two day history of sudden onset of headache and vomiting associated with diplopia and declining vision in the left eye for a few months. On admission he had a high grade fever. Neurological examination was unremarkable except for left sided blindness and blurred vision in right eye. Initial investigations revealed CRP of 181. CT head reported no haemorrhage or infarct. CSF analysis: WCC 24 (polymorphs 60% lymphocytes 40%), protein 0.92, glucose 3.3, no bacterial growth. After further deterioration in the right eye vision and no response to antibiotics and antiviral therapy, an urgent brain MRI was arranged which confirmed a pituitary macroadenoma with suprasellar extension causing compression and stretching of the optic chiasma. Subsequently, the patient underwent a successful transphenoidal debulking of the pituitary lesion. Postoperatively the patient regained partial right eye vision but the left eye remained blind.

Case 2
A 75-year-old type 2 diabetic male presented with a three day history of mild headache, diplopia and drowsiness. Initial investigations: Na 125, K 4.1, glucose 7.7, random cortisol 482, CT head findings consistent with cerebral atrophy. CSF analysis: WCC 1, glucose 4.9, protein 0.80. Within 48h of admission, GCS dropped to 3/5 with flaccid tetraparesis and areflexia. He subsequently developed polyuria and diabetes insipidus. MRI head confirmed pituitary fossa lesion with elevated optic chiasma. GCS improved to 5/5 after initiation of steroids. He also developed a right fifth nerve palsy. He was deemed unfit for neurosurgical intervention. Six months later, he remains stable with steroid and thyrxine replacement.

Conclusion
The diagnosis of pituitary apoplexy is often delayed due to atypical presentation. Due to the serious complications of pituitary apoplexy, timely recognition and appropriate management is critical.

P190
Adrenal incidentalomas: should we be doing PET scans?
Nisha Kaimal, Simon Taggart, Harry Mantora, Stephanie Soteriadou, Helen Doran & Annice Mukherjee
Salford Royal Foundation Trust, Manchester, UK.

A 60-year-old man underwent investigations for weight loss and abdominal pain. A CT thorax/abdomen revealed a 2 cm right adrenal nodule, 3 nodules in the left adrenal <1.4 cm and a 5 mm lung nodule in the right middle lobe. The single phase CT was unable to characterize the adrenal lesions. MRI adrenal showed solid mixed signal pattern in the nodules with signal drop off in opposed phase sequences consistent with adenomas. Twenty-four hour urine catecholamine, cortisol, DHEAS and renin aldosterone levels were normal. GI investigations demonstrated no cause for weight loss. Repeat CT after 6 months showed stable appearances of lungs and adrenals. Adrenal CT at 14 months from initial scan showed an increase in size of 0.5 cm in the right and 0.3 cm in the left adrenal nodules, considered marginal. Four months later the patient presented with chest pain. A chest X-ray demonstrated a new left lung lesion. Staging CT suggested a new left upper lobe lung carcinoma with mediastinal lymphadenopathy. Endobronchial ultrasound guided FNA of an enlarged mediastinal lymph node confirmed a squamous cell lung cancer. Both adrenal glands showed high avidity to FDG on PET-CT imaging. The patient is currently undergoing palliative chemotherapy.

Discussion
This patient developed a new lung cancer after follow-up of presumed benign adrenal incidentaloma. The lung cancer was not visible on initial or early follow-up imaging.

The prevalence of malignancy in adrenal incidentalomas is <5%. In view of the patient’s symptoms and multiple adrenal nodules an underlying malignancy was initially sought but not found, however PET was not performed at that stage. An earlier PET scan may have expedited the diagnosis of malignancy in this case.

The utility, indication and timing of PET scanning in the evaluation of adrenal nodules are pertinent issues raised by this case.

P191
Endocrine neoplastic manifestations of neurofibromatosis type 1 (NF1): a case and discussion
Richard Carroll & Jeannie Todd
Imperial College Healthcare NHS Trust, London, UK.

SB, a 54-year-old male, was diagnosed with NF1 aged 11 on the basis of multiple café au lait spots and neurofibromata. In 1991 he presented with diarrhea and weightloss and investigations revealed a duodenal mass and suspected hepatic metastases. Somatostatin levels were elevated, and histology confirmed a duodenal somatostatinoma following Whipple’s procedure in 1991. The hepatic metastases were excised with good results. At follow up, SB remained symptom free treated only with Creon supplements. An Indium-111 Octreotide scan in 2001 showed normal distribution with no evidence of recurrence. Unfortunately, he represented in 2010 with abdominal pain, jaundice, and diarrhoea. Somatostatin levels increased to 684 nmol/l (NR<150 nmol/l), having been <495 nmol/l during the years of radiological stability. CT imaging revealed recurrent hepatic disease obstructing the common bile duct along with significant paraaortic and mesenteric lymphadenopathy. Gallium 68 DOTATATE PET CT scan revealed no uptake in the lesions and therefore, following MDT discussion, SB has been referred for systemic chemotheraphy.

Neurofibromatosis type 1 was first described by Von Recklinghausen in 1882, and in the various phenotypes were documented 1956. It is a autosomal dominant disorder affecting 1 in 3500 people, characterised by the presence of multiple café au lait spots, skinfold thickening, and neurofibromata amongst other features. Endocrine neoplasia is rare in association with NF1 but phaeochromocytoma, gastrointestinal neuroendocrine tumours, medullary thyroid cancer, and hyperparathyroidism are described. Of these, phaeochromocytoma has the highest frequency at 1-5%. Additionally, optic glomas seen in 15% of children with NF1 are associated with precocious puberty. The diagnosis of NF1 will be discussed based on current guidelines, along with a review of the literature documenting NF1 related endocrine neoplasia. The clinical management of NF1 should include screening for endocrine neoplasia and phaeochromocytomas and NF1 should be considered in the differential diagnosis for patients presenting with these neoplasms.

P192
Familial adrenocortical carcinoma associated with HNPCC
Narayan Kanandasamy1, Serena Nik-Zainal2, Anand Kumar Annamalai3, Lisa Walker2, Lisa C Happerfield1, Mark J Arends1, Joan Patterson2 & Mark Gurnell2
1Institute of Metabolic Science, Addenbrooke’s Hospital, Cambridge, UK; 2Department of Medical Genetics, Addenbrooke’s Hospital, Cambridge, UK; 3Department of Histopathology, Addenbrooke’s Hospital, Cambridge, UK; 4Department of Clinical Genetics, Oxford Radcliffe Hospitals, Oxford, UK.

We report the first case of familial adrenocortical carcinoma (ACC) in association with hereditary non-polyposis colorectal cancer (HNPCC) in a family with a MSH2 germline mutation.

HNPCC, an autosomal dominant disorder caused by mutations in one of the DNA mismatch repair (MMR) genes, is the commonest cause of hereditary colon carcinoma, and is associated with an increased risk of certain non-colonic cancers (e.g. endometrial, ovarian, urinary tract, biliary tract). Currently, ACC is not a recognised feature of the HNPCC syndrome, and there are only two previous reports of ACC arising in the context of HNPCC, both of which were non-familial tumours. The Amsterdam II/Revised Bethesda criteria are used to select patients, based on family history of colorectal cancer or other HNPCC-associated tumours, for immunohistochemical (IHC) analysis to detect loss of MMR protein expression, or for microsatellite instability (MSI) in the tumour DNA. Those with loss of MMR expression or MSI are offered mutation analysis of the four MMR genes. The proband presented aged 54 years with generalised abdominal pain. Her past medical history included ovarian cancer (age 44 years) and a malignant colon polyp (age 47 years). Investigation showed an extensive right adrenal mass, which was surgically resected. Histology confirmed an ACC with capsular, vascular and lymphatic invasion. Review of the family history revealed several individuals with colorectal cancer and other HNPCC-associated tumours. In addition, her mother had died of metastatic ACC. IHC analysis demonstrated loss of MSH2 protein expression in both the ACC from the proband and her mother. Mutation analysis confirmed a germline MSH2 mutation (deletion of exons 1–3) in the proband and several other family members.

Endocrine Abstracts (2011) Vol 25
The familial occurrence of a rare tumour in this HNPCC family, strongly argues for a causal link with the underlying MMR defect.

P193
Prevalence and follow-up of adrenal incidentalomas after CT renal colic
Olympia Koulouri, Lisa Turner, Giridhar Tarigopula & Marie-France Kong
University Hospitals of Leicester, Leicester, UK.

Introduction
CT renal tract is commonly requested by the urologists for suspected renal colic as it is recognized as the most accurate technique for the detection of ureteric stones. However, follow-up of adrenal incidentalomas identified on such scans could pose a challenge for the non-endocrinologist. We investigated the prevalence and follow-up of incidentally discovered adrenal masses after CT renal colic.

Methods
We looked through the reports of all CT renal tract during a 12-month period to identify adrenal incidentalomas. We then sought evidence of further investigations and follow-up.

Results
Eight hundred and sixty-three scans were identified. Nine patients (1%) were found to have adrenal incidentalomas. Median age was 60 years (range 40–75 years). Seventy-eight percent of the patients were male. In 2 cases malignancy with adrenal metastases was diagnosed on further contrast enhanced imaging. In a further case a contrast CT revealed no adrenal abnormality. One patient with a left sided 13 mm adrenal mass is awaiting further imaging and 1 with a 16 mm mass had has a negative screen for urinary catecholamines under the urologists but no other follow-up. The remaining 4 patients have not had any follow up arranged.

Conclusion
The abnormal adrenal findings were not included in the discharge summaries. None of the 9 patients have been referred to the endocrine clinic.

P194
Bowel obstruction can be the presenting symptom of pheochromocytoma
Anna de Lloyd, J Stephen Davies & David Scott-Coombes
University Hospital of Wales, Cardiff, UK.

We describe the case of two patients who presented with non-mechanical bowel obstruction as a consequence of an underlying, undiagnosed Phaeochromocytoma. The first patient was referred in to the surgical team by GP with signs and symptoms of small bowel obstruction. He described abdominal pain, distension and vomiting and had not opened his bowels for a week. The X-ray supported the clinical diagnosis and he went on to have an abdominal CT scan. The scan did not identify an obstructing mass however it did reveal a unilateral 10 cm adrenal tumour. Urine catecholamines were consistent with the diagnosis of phaeochromocytoma. Alpha-blockers were introduced with prompt restoration of the patient's condition.

The second patient was referred for a surgical opinion on her 8th post-operative day. Her abdomen was soft but tender and she appeared to be distressed. On account of a progressive deterioration in her clinical parameters; tachycardia, tachypnoea and progressive lactic acidosis a CT scan was arranged. Her abdomen was soft and tender but did confirm a locally infiltrative adenoma. She was taken to theatre for a curative resection. The post operative period was uneventful.

P195
P53 is induced by ionising radiation and functionally inactivates p53 in thyroid cancer
Robert Seed, Martin Read, Jim Fong, Greg Lewy, Vicki Smith, Perkin Kwan, Gavin Ryan, Kristien Boelaert, Jayne Franklin & Chris McCube
University of Birmingham, Birmingham, UK.

PTTG is a multifunctional proto-oncogene overexpressed in thyroid cancers, which binds to p53 and modulates its function. PBF, a binding partner of PTTG, is also overexpressed in thyroid cancer and can transform cells independently of PTTG. Moreover, subcutaneous expression of PBF elicits large tumours in nude mice. Given the established role of ionising radiation in thyroid tumourigenesis, we investigated the relationship between PBF and the tumour suppressor protein p53. PBF repressed p53-mediated gene regulation through HD-M2 promoter assays in p53-null H1299 cells. Transfer of p53 elicited a 143 ± 17-fold stimulation of promoter activity, whereas co-transfection of PBF significantly repressed p53 transcriptional activity (41 ± 5-fold, P < 0.001). Exposure of wild-type mouse thymocytes to gamma-irradiation resulted in a significant increase in PBF protein expression after 24 h (1.88 ± 0.09-fold, P < 0.017). Co-immunoprecipitation assays revealed direct binding of PBF and p53 in TPC-1 human thyroid papillary carcinoma cells, with a marked increase in binding after treatment with gamma-irradiation. Furthermore, transient overexpression of PBF in TPC-1 cells resulted in a significant decrease in p53 protein levels as compared to controls (75 ± 2.5-fold decrease after 90 min, P < 0.028, n = 4). Finally, in MTT proliferation assays, we observed a significant reduction of cell viability in mock-transfected TPC-1 cells after treatment with gamma-irradiation compared to untreated controls (7.9 ± 0.008% decrease, P < 0.016, n = 4). Critically, overexpression of PBF abrogated this observed decrease of cell viability (0.2 ± 0.007%, P = NS, n = 4). Taken together these data highlight a novel potential mechanism of thyroid tumourigenesis, whereby PBF stabilises in response to DNA damage, binds directly to p53 and inhibits its function.

P196
MEN-1 mosaic: the founder of a family
Selene Farook, Daniel Kannapan, Sami Kenz, Fiona Lalloo, Peter Trainer & Georg Brabant
Christie Hospital, Manchester, UK.

Multiple endocrine neoplasia 1 (MEN-1) is an inherited autosomal dominant tumour syndrome affecting mainly the parathyroid gland, pituitary and pancreas. Genetic defect appears to be deletion mutation of MEN1 gene coding for tumour suppression. We describe a case of MEN1 mosaic mutation never reported in the literature.

The index case presented aged 52 in 1985 with headaches and dizziness when hypercalcaemia of 3.2 mmol/l was noted. Past medical history included stable ulcerative colitis, duodenal ulcers, renal stones and arthritis. In 1990 she had excision of an enlarged parathyroid gland and histology confirmed benign adenoma. She required further neck exploration surgeries in 1992 and 2002 for persistent hypercalcaemia and recurrence of adenoma.

When her daughter developed hypercalcaemia due to a parathyroid adenoma, we investigated the family for familial hyperparathyroidism. Mutation testing of the index case identified low level mosaicism for 188delG mutation of MEN1. This is likely to have arisen due to a sporadic mutation in a single cell of a 4-8 cell embryo, resulting in only a proportion of cells with the mutation. Genetic testing on her daughter confirmed heterozygous for 188delG mutation confirming germline mosaicism in index case.

Survelliance imaging of the index case in 2008 revealed a pituitary microadenoma and two pancreatic lesions (2.5 × 2 cm, 1.1 × 0.7 cm) with normal serum fasting gut peptides and pituitary function. Serial followup imaging showed stable appearances. Calcium levels remain normal.

The family described is unique for MEN1 showing a founder effect in the index case due to mutation of single cells very early in development. Mosaicism is a recognised mechanism in a number of other inherited cancer syndromes but not reported in MEN-1 to date. We could argue that a lower dose of mutated protein could explain the relatively milder clinical presentation which nevertheless leads to a classical pattern of tumour formation.

Endocrine Abstracts (2011) Vol 25
A 33-year-old lady was referred to the endocrinology clinic with weight gain, hirsutism and amenorrhea. She had been diagnosed with hypertension a year ago which was difficult to control despite being on three anti-hypertensive agents – Ramipril, Amlodipine and Bendroflumethiazide. Past medical history included hypertension secondary to radioactive-iodine therapy for Graves disease aged 22. Her GP organised an ultrasound scan querying polycystic ovaries but this revealed a large left adrenal mass.

In the endocrine clinic her history was suggestive of pheochromocytoma with paroxysms of chest pain, palpitations and a feeling of impending doom. On examination however, she appeared cushingoid with hirsutism, central obesity, abdominal striae and proximal myopathy. She had no history of headaches and visual fields were full on confrontation. There was no family history of clinically overt endocrine disease.

Results

Urinary free Cortisol 260 and 424 mmol/l (normal 10–147), failure to suppress on both low and high dose dexamethasone tests, ACTH <5 ng/l (0–47), corrected calcium 2.82 mmol/l (2.1–2.6), PTH 18.1 pmol/l (1.5–7.6), 24 h urine calcium 18.4 mmol/l (2.5–7.5), two sets of 24-h urine catecholamines were normal. MRI Adrenals showed an 8 cm left adrenal mass. Neck ultrasound showed a right inferior lobe parathyroid adenoma.

She underwent laparoscopic adrenalectomy followed by parathyroidectomy a few months later. Histology showed a benign adrenal cortex adenoma and a parathyroid adenoma respectively. Interestingly soon after the adrenalectomy the hypercalcaemia got worse (levels rising above 3 mmol/l) and symptomatic that required intravenous fluids and bisphosphonate treatment.

Four months post surgery she had lost 10 kg in weight and stopped her anti-hypertensive treatment. She remains on hydrocortisone replacement.

This case highlights the rare concomitant presentation of adrenal Cushings’s and hyperparathyroidism as well as the unusual association of Graves disease in the same patient. It also reminds us the importance of screening for secondary causes in young hypertensive patients.

Conclusion

P198

PTTG and PBF: targets for enhancement of radiodiode uptake in thyroid tumours

Gregory Lewy, Martin Read, Vicki Smith, Fong Jim, Gavin Ryan, Sarah Hart, Robert Seed, Neil Sharma, Perkin Kwan, Margaret Eggo, Jayne Franklyn, Christopher McCabe & Kristien Boelaert

University of Birmingham, Birmingham, UK.

Iodide uptake via the sodium iodide symporter (NIS) is reduced in many thyroid cancers, resulting in poor prognosis following treatment with 131I. The pituitary tumour transforming gene (PTTG) and its binding factor (PBF) are proto-oncogenes implicated in thyroid tumourigenesis and we previously demonstrated PTTG and PBF-induced repression of NIS in vitro. We have recently generated murine transgenic models of targeted overexpression of PBF and PTTG in the thyroid to investigate their function in vivo. Thyroid glands were harvested from 6-week old age- and gender-matched wild-type (WT), PBF and PTTG transgenic mice for mRNA analysis, immunohistochemistry or primary murine thyroid cultures. PBF and PTTG overexpression were confirmed in PBF and PTTG transgenic mice by qPCR. Subsequent functional studies demonstrated enhanced uptake of 125I in primary PBF (77.6±10.2% reduction, P=0.0004, n=6), PTTG heterozygote (43.4±7.6% reduction, P=0.042, n=15) and PTTG homozygote (65.4±9.9% reduction, P=0.004, n=14) mouse thyroids compared with WT controls. Following transfection with a PBF-specific siRNA, iodide uptake was restored in PBF thyroid cultures (2.4±0.64 fold increase, n=20, P=0.04) compared with scrambled siRNA controls, with uptake levels indistinguishable from those in WT thyroid cultures (P=0.86).

Conclusion

PTTG and PBF potently repress NIS expression and function in vivo. Studies using PBF transgenic mice and human primary thyroid cultures emphasise PBF as a critical regulator of NIS function, and implicate PBF as a therapeutic target to improve treatment with 131I in thyroid tumours.
Ultrasound of the neck showed a possible left superior parathyroid adenoma and sestamibi was unsurprisingly negative. An MDT discussion based on the options of surgery or watchful waiting fell temporarily in favour of the latter. However, several months later axillary calcium remained high despite thiazide therapy and cinacalcet was able to suppress the plasma calcium to the lower half of the normal range (2.17 mmol/l) suggesting a relatively large parathyroid component to the hypercalcaemia. A DEXA scan also demonstrated lumbar osteopaenia. The patient went onto have an uncomplicated 4 gland exploration of the parathyroids. The left superior parathyroid gland appeared abnormal to the naked eye and reported histologically as a parathyroid adenoma confirming our original diagnosis.

Insulinoma presenting as post-prandial hypoglycaemia
Asgar Madathil1 & Jolanta Weaver2
1Gateshead Health NHS Trust, Gateshead, UK; 2Faculty of Medical Sciences, Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK.

Although insulinoma commonly presents as fasting hypoglycaemia it can rarely present as post prandial hypoglycaemia and even less rarely in association with Type 2 Diabetes Mellitus (T2DM). We describe a case of insulinoma presenting as post-prandial hypoglycaemia and T2DM.

A 60 year old man presented with a 6 year history of episodes of double vision and was referred to the ophthalmology clinic in 2006. Further investigations at this time were unremarkable. He was given dietetic advice for post-prandial hypoglycaemia and was followed up routinely.

After 2 years, hypoglycaemic symptoms worsened by exercise and delayed meals. A number of supervised fasting glucose measurements failed to demonstrate biochemical hypoglycaemia. His insulin, c-peptide and pro-insulin levels were mildly elevated but plasma glucose levels were normal. A CT scan showed 20 mm hypervascular lesion in the distal pancreas which was confirmed as insulinoma with pancreatic arterial calcium stimulation studies. Laparoscopic resection confirmed benign insulinoma and hypoglycaemia resolved. A repeat OGTT after 12 months showed T2DM and at 18 months impaired glucose tolerance.

Persistence of hypoglycaemic symptoms should always be taken seriously. Our case illustrates the importance of considering insulinoma as a cause of post-prandial hypoglycaemia. A high index of suspicion in patients with post-prandial hypoglycaemia, who do not respond to conventional treatment or whose pattern of symptoms change will lead to further investigations.

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia: a precursor to pulmonary carcinoid
Mark Stephens & Aled Rees
University Hospital of Wales, Cardiff, UK.

Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia (DIPNECH) is a rare pulmonary pathology which encompasses a spectrum of findings ranging from simple neuroendocrine cell proliferation to discrete nodules, and is strongly associated with carcinoid tumours. Patients, typically female, are often asymptomatic, but may present with overt pulmonary symptoms, such as dyspnoea, cough or pleuritic chest pain; however, even in their absence, a degree of obstructive or mixed obstructive/restrictive deficit on formal pulmonary function testing is common.

We describe the case of a 60 year old female non-smoker who initially presented with a chronic non-productive cough and right subcostal pain. Whilst obese and hypertensive, pulmonary imaging and endocrine function were generally unremarkable, though both FSH and LH were inappropriate low for a post-menopausal woman. A single discrete 2 cm mass in the right upper zone was accompanied by diffuse nodularity throughout both lung fields on CT scanning. Biopsy showed the mass to be a low grade spindle cell carcinoid tumour. Subsequent Video Assisted Thoracic Surgery confirmed the presence of neuroendocrine tumourlets and multiple foci of neuroendocrine cell hyperplasia in the contralateral lung. Ga-Octreotate-PET scanning failed to demonstrate any uptake in either the tumour or pulmonary nodules, suggesting no significant somatostatin receptor expression. The patient underwent a right middle lobectomy, and has remained symptomatically well since the procedure. Interestingly, a single subcarinal lymph node showed minimal tracer activity during PET scanning, whilst histologically metastatic spread to a single lymph node was reported.

Although perhaps typical in its presentation, we feel that this case merits reporting, in the hope that it will promote further discussion about the management and surveillance of DIPNECH.

Five year experience of investigation and management of historically proven chromaffin cell tumours
Anjali Amin, Fausto Palazzo, Karim Meeran & Jeannie Todd
Imperial College Healthcare NHS Trust, London, UK.

Chromaffin cell tumours are rare but potentially curable endocrine tumours. These tumours may be sporadic or familial in nature. Biochemical tests are normally performed initially, followed by radiological investigation.

To assess the correlation of biochemical and radiological investigations with historically proven chromaffin-cell tumours in patients with sporadic and familial disease.

We retrospectively reviewed data for 28 patients who underwent adrenalectomy for presumed chromaffin cell tumours between October 2005 and October 2010. Results
18 of 28 patients underwent laparoscopic adrenalectomy, 5 patients had MEN2A, 2 had NF1, 1 had VHL and 2 were SHDB + ve. Histology confirmed pheochromocytoma in 19, 1 of which was malignant and 5 parangangiomas, 1 of which was malignant. Biochemical data were available for 21 patients. Pre-operative 24-hour urine catecholamines showed mean adrenaline 1.51 ± 1.38 µmol (NR 0.00–0.10), mean noradrenaline 3.05 ± 1.44 µmol (NR 0.00–0.50), and mean dopamine 2.10 ± 0.41 µmol (NR 0.00–2.70). All patients with MEN2A, NF1 and VHL had negative urine catecholamines. Both patients with SHDB mutations had positive urine catecholamines. Two of the patients with MEN2A had urine metanephrines performed, one of which was positive despite negative urine catecholamines. Similarly, one of the NF1 patients had positive urine metanephrines with negative urine catecholamines. MiBG scan was positive in 18 of 20 patients: of the two patients with negative MiBG, one was also negative on Gallium-68 DOTATATE scan. One of the patients with MEN2A had a negative MiBG scan.

Conclusion
Our results show that urine catecholamines are poor markers in the detection of pheochromocytoma in patients with familial syndromes. We have shown that the sensitivity of urine metanephrines is superior to that of urine catecholamines in patients with MEN2A and NF1. In addition, there are limitations to MiBG scanning; care needs to be taken in interpreting these scans in patients with a genetic predisposition to pheochromocytoma.
towards the identification of biomarkers that may determine treatment decisions.

...52 fold. The pathway protein, Secreted frizzled-related protein 4 (SFRP4), was upregulated in association with 'cellular growth and development' (P significance).

...of 301 genes, 15 genes associated with 'skeletal and muscular function' showed the greatest differential variability in gene expression clustered by cell line and genes in pathways biological function of genes.

...expressed using Affymetrix HG-U133 plus 2.0 arrays, differential gene expression was analysed using ANOVA and Ingenuity Pathway Analysis software (IPA) was used to assess biological function of genes.

...of all the pathways associated with 'skeletal and muscular function' showed the greatest differential expression (35 out of 301 genes, P < 0.0001) with insulin-like growth factor binding protein 5 (IGFBP5) upregulated in TS (670-fold). When the T1 and T5 cell lines were compared, 8 out of 25 differentially expressed genes were associated with 'cellular growth and development' (P < 0.0001) and a Wnt pathway protein, Secreted frizzled-related protein 4 (SFRP4), was upregulated in T1 52-fold.

Identification of GH-dependent growth pathway genes is an important step towards the identification of biomarkers that may determine treatment decisions.

P206
Effects of the prenatal environment on haematological and skeletal muscle parameters in one-week-old piglets: a role for glucocorticoids?...growth of 112 piglets in seven-day-old offspring exposed to suboptimal gestational environments.

...changes in maternal dietary intake during gestation can affect muscle development and may be linked to the catabolic actions of hormones, such as glucocorticoids, which inhibits the insulin like growth factor 1 (IGF1) pathway. This study examines the potential effects of glucocorticoids and skeletal muscle adaptations in seven-day-old offspring exposed to suboptimal gestational environments.

Pregnant sows were randomly assigned to a commercial diet (L/H n = 8), which increased in energy content during gestation, or an inverse diet (H/L n = 8). H/L offspring were not allowed to birth weight as small (SL/H, n = 7) or median (CL/H, n = 7). Only the H/L median offspring (M sl/H, n = 8) were selected for this study. Blood samples were taken from week-old piglets before they were euthanased. The biceps femoris was sampled for histological analysis and real-time gene expression of IGF1, 11β-hydroxysteroid dehydrogenase (HSD)-1 and 2, glucocorticoid receptor GR.

Sodium and creatinine plasma concentrations increased in SL/H offspring compared to the controls. The serum protein rose in both intra-uterine compromised groups in relation to M sl/H (SL/H 49.83 ± 3.1; CL/H 31.00 ± 4.8; M sl/H 43.04 ± 4; g/l (*P < 0.05)). The GR and 11β-HSD-1 genes were upregulated in H/L offspring in relation to the control group, whilst IGF1 expression (SL/H 0.96 ± 0.3; CL/H 1.0 ± 0.1; M sl/H 2.2 ± 0.4; *2.52±0.7t(P < 0.05) and 11β-HSD-2 was highest in M cl/H, compared to the other groups, with more internal nuclei fibres than the SL/H.

Our study suggests that IGF1 and 11β-HSD-2 gene expression in M sl/H offspring muscle increase in response to the reduced maternal calorie intake during the second half of gestation. Increased numbers of fibres with central nuclei may indicate glucocorticoid activation. Further investigations will determine whether this promotes a degenerative or regenerative process.

This study was supported by the BBSRC (BB/H002650/1)

Reference

P207
Genetic characterisation of primary GH Insensitivity (GHI) presenting as growth failure: 10 years experience at the Centre for Endocrinology, William Harvey Research Institute, Barts and the London...and reported the first pyropyrinidine tract mutation. Other unusual cases included a

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Number of individuals</th>
<th>Country of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S40L</td>
<td>7</td>
<td>Turkey</td>
</tr>
<tr>
<td>V125A</td>
<td>2</td>
<td>Iraq, UK</td>
</tr>
<tr>
<td>R161C</td>
<td>1</td>
<td>Israel</td>
</tr>
<tr>
<td>G223G</td>
<td>2</td>
<td>Bahamases, Spain</td>
</tr>
<tr>
<td>L229P</td>
<td>1</td>
<td>UK</td>
</tr>
<tr>
<td>R43X</td>
<td>7</td>
<td>Turkey</td>
</tr>
<tr>
<td>C48X</td>
<td>2</td>
<td>Argentina</td>
</tr>
<tr>
<td>Q65X</td>
<td>1</td>
<td>Turkey</td>
</tr>
<tr>
<td>E180X</td>
<td>1</td>
<td>Mexico</td>
</tr>
<tr>
<td>Q216X</td>
<td>1</td>
<td>Turkey</td>
</tr>
<tr>
<td>IV52 ds +1 G to A</td>
<td>2</td>
<td>Turkey</td>
</tr>
<tr>
<td>IV52 ds +1 G to A</td>
<td>1</td>
<td>Hong Kong</td>
</tr>
<tr>
<td>Pseudoexon insertion</td>
<td>15</td>
<td>Pakistan</td>
</tr>
<tr>
<td>IV57 as-6 T to A</td>
<td>1</td>
<td>Bangladesh</td>
</tr>
<tr>
<td>IV58 as-6 G to A</td>
<td>1</td>
<td>UK</td>
</tr>
<tr>
<td>IV59 as-6 T to C (heterozygous)</td>
<td>2</td>
<td>Spain</td>
</tr>
<tr>
<td>c.1323_1344del22 (450X)</td>
<td>2</td>
<td>Spain</td>
</tr>
<tr>
<td>STATA5B</td>
<td>2</td>
<td>Saudi Arabia</td>
</tr>
<tr>
<td>c.1680delG</td>
<td>1</td>
<td>UK</td>
</tr>
<tr>
<td>IGALFS</td>
<td>1</td>
<td>UK</td>
</tr>
<tr>
<td>P73L</td>
<td>1</td>
<td>UK</td>
</tr>
<tr>
<td>L113C-546-548delGG-</td>
<td>1</td>
<td>UK</td>
</tr>
<tr>
<td>CinsAG</td>
<td>1</td>
<td>Kurdish</td>
</tr>
<tr>
<td>c.1490insT</td>
<td>1</td>
<td>Turkey</td>
</tr>
<tr>
<td>D449N</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Table 1

Endocrine Abstracts (2011) Vol 25
In conclusion, genotyping of candidate genes was informative in the investigation of a significant proportion of patients with GHI. Most cases were caused by defects in GHR but mutations in other genes of the GH-IGF1 axis such as STAT5B and IGFALS are being increasingly recognised as causal.

Summary

The LDDT is a simple, well-tolerated test. It has allowed confident exclusion of tumourous causes of hyperandrogenism, and dramatically reduced the need for further complex investigation. In the light of 1/34 significant anomalous result, we propose that a normal result requires suppression of testosterone levels by both >40% and into the normal range in future.

---

**P208**

The use of a novel injection delivery system to address compliance in patients receiving GH replacement therapy

Stephen McGlynn, Michael Edwards, Linda Smethurst & Georg Brabant

The Christie, Manchester, UK.

The majority of adult GH replaced patients reach target IGF1 levels using established injection devices. However, a small but significant cohort of these patients fail to achieve these intended goals. This can be manifested as either a failure to improve (biochemically or symptomatically) despite escalating GH doses, or large variations in IGF1 measurements despite steady GH dosing. The EasyPod® device has a memory chip which allows recording of time, number and dose of GH injections given.

Ten patients from the cohort described above were invited to trial the EasyPod device, and were standardly trained and followed up by a dedicated nurse. IGF1 levels were monitored as per usual clinical practice both before and after commencing the novel device.

The device was well tolerated, with 8/10 patients remaining on the device at the time of submission. Mean duration for use of the device was 191 days (19–3976). Two patients discontinued use of the device due to mechanical failure and difficulty preparing the injection respectively.

Three patients were identified with compliance issues missing over 20% of doses, with a mean compliance of 89% (range 68–99%). Adequate compliance as evidenced by the administration history in the other patients formed the basis for further dose adaptation to reach target levels which were achieved in 9/10 patients.

Particularly with current uncertainties around IGF1 measurements the down-loadable injection history enables clinicians to draw more accurate conclusions regarding the validity of the IGF1 result. This may have potential benefits in reducing unnecessary dose escalations and allowing to identify non-compliance at an early stage.

---

**P210**

Care of cancer survivors: the role of endocrinologists

Diana Greenfield, Andrew Toogood

Teaching Hospitals NHS Foundation Trust, Sheffield, UK; University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK.

Background

Increasing numbers of children and adults are surviving cancer and living with the consequences of their disease or its treatment. The commonest long term consequences observed following childhood cancer are endocrine and there is a growing body of evidence indicating that adults treated for malignant disease are also at risk of endocrine dysfunction.

Aim

To determine the involvement of Endocrinologists in the management of cancer survivors and resources that will improve the outcome of this growing patient cohort.

Methods

Clinical members of the Society for Endocrinology were invited to complete an online questionnaire.

Results

One hundred and ten physicians (64% male) responded of whom 60.9% were involved in the care of cancer survivors. Respondents cared for patients treated during childhood (18%), adulthood (20%) or both (62%), but only 31% had a dedicated late effects clinic. 97% felt the Endocrinologist should be part of the late effects MDT and 92% agreed that standardised guidelines and specialist nursing support would help them provide a clinically appropriate late effects service. 86% of Endocrinologists felt they would benefit from specific training in endocrinology.

Discussion and recommendations

This survey demonstrates that Endocrinologists are engaged in the care of cancer survivors although provision is not universal. The survey has identified the following needs: development of guidelines for the management of late effects of treatment for cancer in adulthood and standards of care, links with cancer networks; attendance of Endocrinologists at late effects MDTs when established.

There is a particular need to establish training for Endocrinologists who are keen to be involved in the care of these patients.

---

**Nursing practise**

**P209**

Audit of low dose dexamethasone suppression test to exclude androgen secreting tumours in hyperandrogenic women

Katherine Powell, Rosemary Temple & Francesca Swords

Norfolk and Norwich University Hospital, Norwich, UK.

The low dose dexamethasone suppression test (LDDT) is used routinely to exclude Cushing's. This test can also be used to exclude androgen secreting tumours in females with elevated testosterone levels through normalisation of, or >40% suppression of serum levels.

We have audited the use of the LDDT to assess its value in investigating women with raised androgens, to ascertain whether it reduced the need for other investigations and to identify any problems encountered in performing the test.

Method

We reviewed the notes and results of all female patients referred to the clinical investigation unit for LDDT investigation of elevated androgen levels.

Results

26/34 patients suppressed their testosterone levels by >40%. 6/34 suppressed by <40%, all of whom underwent further investigation. 50% were found to have a tumourous cause for the hyperandrogenism. 1/26 patient suppressed by 43% but her testosterone levels remained elevated (5.3 nmol/l). A repeat LDDT resulted in 33% suppression. Further investigation is underway to rule out a tumourous cause in this case.

Two patients having the LDDT for the investigation of hyperandrogenism were found to have non-suppressible cortisol. Cushing's syndrome has subsequently been confirmed in both cases. No adverse events were encountered in any patient.

---

**P211**

Nebido (testosterone undecanoate) in patients over 60 years of age: a time to reduce dose frequency?

Diana Mantripp, Rachel Franklin, John Wass & Niki Karavitaki

Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK.

Background

Clinical experience has shown that men over 60 years of age frequently (40%) require extended intervals of greater than 12 weeks between administrations of Nebido. No work has assessed whether this is specific to men over 60 years of age.

Methods

We analysed men on Nebido over 60 years (n = 12, mean age 66 years) and compared them with a BMI matched group aged 40–60 years. We assessed testosterone levels, injection intervals, PSA and haematocrit over the first 4 injections.

Results

Baseline mean serum testosterone in the over 60 age group was 4.13 nmol/l (range 1.5–12.1) and in the 40–60 age group was 5.85 nmol/l (range 0.4–16.1). Over 60 age group 3 out of 12 patients had an elevated trough testosterone pre-2nd injection (21.3, 22.2, 24.2 nmol/l) and had next injection interval extended to 15, 15, 24 weeks respectively.
4 out of 12 patients had elevated trough testosterone levels pre-3rd injection (23.9, 17.2, 17.5, 17.8). Two of these patients stopped Nebido due to elevated testosterone levels and polycythaemia. One patient stopped due to interval extending greater than 24 weeks.

1 out of 9 patients who continued with Nebido had an elevated trough testosterone level pre-4th injection (18.9 nmol/l) and had next injection interval extended to 20 weeks.

3 out of 12 patients developed polycythaemia, 2 requiring venesection.

1 out of 12 patients developed an elevated PSA above the normal range.

40-60 age group

No patients required the injection interval extending. No patients developed polycythaemia. No patients developed a raised PSA.

Conclusions

This is the first study to compare injection frequency and side effects in men over 60 with men of 40-60 years. Of the men aged over 60 years, 33.3% had elevated testosterone levels pre 3rd injection and 25% developed polycythaemia compared to no men aged 40-60. We have shown that the necessity for 12 weekly injections for men over 60 years is significantly decreased. Particularly careful monitoring for polycythaemia in this group is necessary.

P212

Diagnosis denial exacerbated by thyrotoxicosis

Adrianna Omar & Jackie Gilbert

King’s College Hospital, London, UK.

A 45 year old female civil servant presented to primary care for a routine evaluation of her lipid profile. When questioned, she described a history of lethargy and low mood. The patient’s GP noted features of agitation and sweating and organised a range investigations including thyroid function tests. Past medical history included systemic lupus erythematosus, a diagnosis that the patient had experienced difficulty accepting, requiring an extended period of counselling. Thyrotoxicosis was confirmed; T4 32.1 pmol/l, TSH <0.01 mU/l likely secondary to Graves’ disease with elevated thyroid microsomal and TSHR antibodies. The patient was adamant that she had neither discussed nor consented to an assessment of thyroid function. As a result, she persistently expressed denial regarding the diagnosis, initially declining all intervention and subsequently demonstrating non-compliance with thionamide medication.

After six months, the patient agreed to be reviewed by a liaison psychiatrist to explore her anxieties accepting the diagnosis. In conjunction with frequent specialist thyroid nurse contact, reassurance, reminders and encouragement she adhered to a six month course of carbimazole and thyroxine. On becoming euthyroid, the patient was able to achieve a coherent perspective of the disease. She has remained in remission seven months after discontinuation of medical therapy. Patients may experience prolonged difficulties accepting a diagnosis due to psychosocial circumstances, religious beliefs, level of education, cognitive impairment and psychiatric disorders. This is observed more commonly with diagnoses of malignancy and conditions requiring long term management such as diabetes mellitus.

As thyrotoxicosis may be associated with anxiety, depression, cognitive impairment and paranoid ideation, this may exacerbate pre-existing difficulties in rationalising illness. Such patients should be counselled carefully regarding planned investigations with maintenance of thorough documentation.

P213

Cushing’s syndrome due to ectopic ACTH secretion from a hepatic neuroendocrine tumour

Yahya Mahgoub, Mohamed Suliman & Jeff Simmonds

Southport and Ormskirk NHS trust, Southport, UK.

A 44-year-old lady with hypertension and type 2 diabetes of 10 years duration (BMI 47, HbA1c 9.1%) treated with metformin and exenatide presented with a few days history of feeling generally unwell, vomiting and confusion. Initial biochemical showed pulse 120 per min, BP 115/74 mmHg, normal temperature and multiple abdominal striae. Admission investigations showed Na 140, K 1.4, urea 3.9, creatinine 74, glucose 26, CRP 23 Hb 16.2, WBC 11.6. ABGS on room air showed pH 7.41, PCO2 3.5, PaO2 11.0. She was initially treated with intravenous KCl and received a total of 280 mmol in the first 48 h. On day 2 the potassium improved only to 2.4 but she developed evidence of sepsis (temp 39°C) and metabolic acidosis (pH 7.08). She was treated in ITU with IV antibiotics. Random cortisol back at 2712 nmol/l with ACTH 50 pmol/l. Pituitary MRI was normal while CT of chest, abdomen and pelvis showed a bulky left adrenal gland and two focal liver lesions suggestive of metastasis but no primary tumour detected. A liver biopsy was eventually performed which showed a poorly differentiated large cell neuroendocrine carcinoma. The patient had a stormy hospital stay complicated by severe sepsis, DVT, C. Difficile diarrhea and acute renal failure needing haemofiltration. She died after 6 weeks stay in ITU.

Metastatic neuroendocrine tumours causing ectopic Cushing’s syndrome are rare and as illustrated in this case these tumours can be very aggressive and have a very poor prognosis.

P214

An unusual complication in a patient with Graves’ disease

Ullal Ananth Nayak, Yasmeen Khalid, Ananth Viswanath, Abdul Rashheed Mohamed Zahir, Baldev Malkiat Singh & Harit Narendra Buch

New cross hospital, Wolverhampton, UK.

Introduction

Hyperthyroidism secondary to Graves’ disease is well-recognised to be associated with non-thyroidal immunological manifestations like ophthalmopathy and pretibial myxoedema. We report a patient with Graves’ disease who presented with an unusual complication.

Case

A 68 year old Caucasian lady presented with typical features of hyperthyroidism which was confirmed by free T4 72.0 pmol/l (12.0–22.0 pmol/l) and TSH <0.01 mU/l (0.27–4.20 mU/l). Graves’ disease was confirmed by diffusely increased uptake on radio-nuclide scan and TSH receptor antibodies level of 13 U/l (normal <1.0 U/l). She was commenced on carbimazole and beta-blockers. Shortly thereafter she was admitted to hospital with shortness of breath and atrial fibrillation with a fast ventricular rate. She had a loud pericardial rub and raised jugular venous pressure. Chest X-ray showed cardiomegaly and mild pleural effusion. An urgent echocardiogram confirmed moderately large global pericardial effusion but there were no features of cardiac tamponade. WCC 20.5, CRP 187, ESR 75, autoantibodies were negative and complement levels were normal. Pleural biopsy showed benign inflammatory changes. Stable euthyroidism was maintained with block and replacement therapy and 6 weeks after commencement of anti-thyroid therapy a repeat echocardiogram confirmed resolution of pericardial effusion and inflammatory markers normalised. Unfortunately pleural effusion was complicated by secondary infection leading to empyema requiring intercostal drainage and antibiotic therapy. Six months later, she remains euthyroid with no serous membrane involvement.

Discussion

We believe that pericardial and pleural effusions were related to Graves’ disease with which they had a strong temporal relationship. Other causes for serous membrane involvement were excluded. Although this is uncommon it has been previously described and inflammatory changes related to an autoimmune process are considered to be involved.

Conclusion

Pleural and pericardial effusions are rare but recognised and serious complications of Graves’ disease and should be considered in patients who present with dyspnoea or chest pain.
the timing of redosing for RAI which varies in different centres between 6 weeks and 12 months depending on the perceived time to respond to therapy.

Aim
We have undertaken a retrospective study to assess if after the initial RAI dose of 400 MBq a second RAI dose can be administered earlier than at 6 months, which is the redosing policy at our centre.

Patients and methods
Retrospective assessment of 199 patients for whom 6 month follow-up data was available. Clinical and laboratory data was obtained from patient notes and electronic database. Cure of hyperthyroidism was defined as achieving euthyroidism or hypothyroidism on the basis on FT4 at FT4 and FT3.

Results
At 6 weeks post-RAI, 70 patients were hyperthyroid, of whom 58 (83%) patients achieved spontaneous cure and 12 (13%) patients remained hyperthyroid requiring a second RAI dose. At the 3 month-stage, 29 patients were hyperthyroid of whom 17 (59%) patients achieved spontaneous cure and 12 (41%) patients required a second RAI dose. For patients who were hyperthyroid at 3 months, a high initial FT4 at diagnosis predicted persistence of hyperthyroidism at 6 months and the need for redosing (74.47 ± 24.65 pmo/l (mean ± s.d.) for patients not cured at 6 months vs 40.19 ± 18.92 pmo/l (mean ± s.d.) for those cured, \( P<0.001 \)). Persistence of hyperthyroidism was not correlated to any other clinical or biochemical predictors studied.

Conclusion
Following the administration of 400 MBq RAI, a significant proportion of patients who were hyperthyroid at 6 weeks and 3 months, revert to euthyroidism by the end of 6 months. In view of this an earlier use of second dose cannot be recommended as a routine measure although this may be considered in patients with high FT4 at diagnosis.

P217
Use of plasma metanephrine estimation in the diagnostic work-up of phaeochromocytoma: an audit of local practice
Marco-Daniel Egawhary1, Yasmeen Khalid2, Varadarajan Baskar2, Rousseau Gama1 & Harit Narendra Buch2
1Birmingham Medical School, Birmingham, UK; 2Department of Endocrinology, New Cross Hospital, Wolverhampton, UK; 3Department of Clinical Chemistry, New Cross Hospital, Wolverhampton, UK.

Background
Urinary catecholamine measurement has been the mainstay for diagnosis of a phaeochromocytoma. Plasma meta-nephrine estimation has been introduced more recently although its precise position remains unclear. We have undertaken a retrospective analysis of patients who have undergone this test to assess its usefulness in the diagnostic process.

Patients and methods
We evaluated all patients who have had plasma metanephrine estimation over the past 4 years. The pre-test clinical and biochemical information and patient outcomes at a minimum of 2-year follow up was correlated with the test result. We also evaluated clinical and biochemical profile of the last 10 consecutive patients who underwent surgery for suspected phaeochromocytoma.

Results
Twenty-three patients had plasma metanephrine estimation. Four patients had typical clinical features with normal multiple urinary catecholamines and 19 patients had raised one or both catecholamines of <3 times upper limit of reference range (ULRR). 22/23 had normal plasma metanephrines. All patients have been stable over a 2-year follow-up period excluding phaeochromocytoma. 1/23 had raised plasma metanephrines with unequivocally positive imaging studies but was unfit for surgery. During the same period, 10 patients had surgery for excision of a suspected phaeochromocytoma. None of these patients had plasma metanephrines estimation. 9/10 had a strong clinical suspicion and urinary adrenaline or noradrenaline >3 times ULRR in at least one urine sample. Phaeochromocytoma was confirmed histologically in all patients. 10th patient had surgery without biochemical confirmation due to an increase in size of adrenal incidentaloma. Histological examination excluded phaeochromocytoma.

Conclusion
Our results confirm that plasma metanephrines estimation has a high negative predictive value in the diagnosis of a phaeochromocytoma. This test does not appear to have a significant role in patients with high clinical suspicion and unequivocally raised urinary catecholamines. This selective approach would avoid unnecessary use of this expensive andlogically difficult test.
P219

Low bone mass is an infrequent long-term sequela of pituitary disease
Julie Lynch & Robert Murray
Leeds Teaching Hospitals NHS Trust, Leeds, West Yorkshire, UK.

Introduction
Within the setting of putative or established pituitary disease the primary disease process (i.e. Cushing’s disease), hormone deficits (i.e. sex steroids, GH) and inappropriate replacement therapy (i.e. glucocorticoids) are reputed to predispose to low bone mass.

Patients and Methods
We examined bone mass at the lumbar spine (LS) and total hip (TH) using DXA in 259 patients with an insult to the hypothalamo–pituitary axis (51.6 ± 15.7 years; 133F; BMI 29.3 ± 5.5 kg/m²). Mean duration of follow-up 11.4 ± 9.5 years. In patients who were receiving treatment for osteoporosis the scan result immediately before therapeutic intervention was used in this analysis.

Results
In the cohort overall Z-scores at the LS and TH were +0.09 ± 1.81 and +0.54 ± 1.21 respectively. A Z-score of less than −2.0 was observed at the LS in 10.4% and at the TH in 2.2% of individuals. No difference was observed in bone mass between subgroups at either the LS or TH following stratification for the primary pathology (Table). Further analysis of bone mass (Z-scores) by the number of additional anterior pituitary hormone deficits revealed no evidence of a trend towards lower bone mass with greater degree of hypopituitarism.

Table 1

<table>
<thead>
<tr>
<th>Category</th>
<th>Lumbar spine</th>
<th>Total hip</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFPA</td>
<td>72</td>
<td>+0.136 ± 1.676</td>
</tr>
<tr>
<td>Acromegaly</td>
<td>35</td>
<td>+0.553 ± 1.632</td>
</tr>
<tr>
<td>Cushing’s Dis</td>
<td>21</td>
<td>+0.305 ± 1.879</td>
</tr>
<tr>
<td>Hyperprolactinaemia</td>
<td>39</td>
<td>+0.419 ± 1.436</td>
</tr>
<tr>
<td>Craniosynostosis</td>
<td>21</td>
<td>−0.261 ± 1.515</td>
</tr>
<tr>
<td>XRT-Induced</td>
<td>10</td>
<td>−0.410 ± 1.847</td>
</tr>
<tr>
<td>Others</td>
<td>61</td>
<td>−0.333 ± 2.037</td>
</tr>
</tbody>
</table>

Conclusion
The impact of hypopituitarism and hormone replacement therapy has negligible impact on bone mass in long-term survivors of patients with a putative or established insult to the hypothalamo–pituitary axis.

P220

Joint radiodine clinic – patient satisfaction survey
Srilatha Dampetla1, Waqas Shafiq1, Akrem Elmalti1, David Pears2 & Mohamed Malik2

1Scunthorpe General Hospital, Lincolnshire, UK; 2Northern Lincolnshire and Goole Hospitals NHS Foundation Trust, Lincolnshire, UK.

Background
Radio iodine treatment is widely used in the management of hyperthyroidism. Recent guidelines have stressed the importance of advising patients of the recommendations with regard to radiation protection, and the implications of radionuclide treatment in relation to work, travel and contact with the family. We carried out a survey to assess our joint radiiodine clinic approach where all patients referred for treatment are assessed jointly by a consultant endocrinologist (Practitioner) and Medical Physicist Expert (Operator) to ensure adequacy of information received.

Survey process
A questionnaire was devised and posted to patients who had been given radiioiudine capsule from January 2007 – January 2009. The questionnaire were divided into four main domains: i) Written information received prior to the clinic appointment, ii) Information given at the clinic appointment, iii) Information at the time of the treatment administration, and iv) How happy patients with the information provided and the journey through the radiiodine treatment.

Results
One hundred and fifty patients were sent a questionnaire to complete with 100 (66.6%) returning a completed questionnaire.

P221

Assessment of bone density in patient with adrenal insufficiency
Bluano Varupula, Angelos Kyriacou & Abu Ahmed
Blackpool Victoria Hospital, Blackpool, UK.

In a retrospective study, we studied the medical notes of 35 patients with adrenal insufficiency; aged 61 ± 15 (mean ± s.d.) years. Their average age at diagnosis was 45 ± 18 years, and duration of the disease was 17 ± 15 years.

Most patients were on hydrocortisone replacement (32 patients); taking 23 ± 4 mg daily in divided doses, and only 3 (9%) patients were on single dose of prednisone 7.5 ± 0.5 mg daily in a single dose. Average body weight at diagnosis was 66 ± 15 years, and last body at time of this study was 75 ± 15 years.

Thirty patients (86%) have reduced bone density on Dual-energy absorptiometry (BMD) scan, 14 patients have osteoporosis and 16 had osteopenia. The Z-score of hip and spine were also reduced. There was no difference in the daily hydrocortisone dose in patients with osteoporosis (13 patients), compared to the 19 patients on hydrocortisone with no osteoporosis (22.2 vs 24.2 mg daily). Conversely, when we stratified the hydrocortisone dose cohort into those with total daily dose of 20 mg (17 patients), 25 (9 patients) and 30 mg (6 patients), the prevalence of reduced bone density was 52.9% (9), 33.3% (3) and 16.7% (1) (Pearson χ² 0.54, P=0.54). Among the three patients receiving prednisolone only one had osteoporosis (total daily dose 6 mg).

In conclusion, osteoporosis and osteopenia were very common (85.7%) in those with adrenal insufficiency. No correlation was identified between the dose of steroid replacement and the prevalence of reduced bone density.

Pituitary

P222

Has you appearance changed over the last few years? Oh I’m really ugly now!
Vinay Somashekar Eligar, Thomas Dacruz, Munveer Thind, Srinivas Rao, Meurig Williams & Samuel Rice
Prince Philip hospital, Llanelli, Carmarthenshire, UK.

Forty two year Mrs SJ, community support worker with a background history of hypothyroidism was referred by her GP with excessive tiredness and weight gain. She had been attending her surgery for few months with somatic symptoms and her GP had noticed significant change in facial characteristics and large hands. She complained of increase in her feet size from a size 6 to 9. No disturbance of vision was reported. She was noted to have hyperglycaemia and oral glucose tolerance test confirmed abnormal growth hormone levels. IGF1 was 128 nmol/l and GH levels were >40 μg/l at 0 min and 37.3 μg/l at 120 min. Prolactin level was elevated at 1800 mIU/l. Her synacthen and thyroid function tests were normal. An MRI scan demonstrated a predominantly intrasellar pituitary macroadenoma with no optic chiasm compression.
A week following the biochemical diagnosis of acromegaly she presented with headache, vomiting and fatigue for 2 days. We suspected pituitary apoplexy, however pituitary CT did not show any change in size of the pituitary adenoma nor did it demonstrate any sign of haemorrhage. A synchthon test was again normal. Her symptoms resolved spontaneously after 1 day. She was started on cefuroxime 0.5 mg twice weekly and discharged. Following 4 weeks of treatment her prolactin had normalised but her IGF1 remained elevated (134 nmol/l). She then presented with sudden onset polyuria and polydipsia and was admitted for a water deprivation test that confirmed the diagnosis of cranial diabetes insipidus. Her osmotic symptoms resolved with DDAVP 10 mg twice daily.

A subsequent MRI again showed a pituitary tumour measuring 2×1.5 cm as before but now with a concave superior border indicating shrinkage of tumour. The posterior pituitary bright spot had also disappeared.

The diagnosis here is pituitary adenoma producing GH which has undergone structural change, most likely as a result of haemorrhage or infarction, leading to the development of diabetes insipidus.

### P223

**Mortality in cushing’s disease: stoke-on-trent data and meta-analysis**

Richard Clayton, Jurina Raskauskienė, Rusail Reulen & Peter Jones

1University Hospital of North Staffordshire, Stoke-on-Trent, UK; 2Walsall Manor Hospital, Walsall, UK; 3School of Health and population Sciences, University of Birmingham, Birmingham, UK; 4School of Computing and Mathematics, Keele University, Stoke-on-Trent, UK.

There are very limited data on long-term mortality in pituitary ACTH dependent Cushing’s disease (CD). We report on our data from Stoke-on-Trent, UK, spanning 50 years and provide a meta-analysis of six other reports which addressed mortality of CD. Case records of 60 CD patients from 1958-31 Dec 2009 from Stoke-on-Trent were reviewed. The standardised mortality ratio (SMR) overall and separately for patients in remission and having persistent disease was calculated. Remission was defined as resolution of clinical features and normalisation of biochemical hypercortisolism according to the methods extant at the time within 3 years of diagnosis.

Overall SMR for the whole cohort was 4.8 (2.8–8.3 95% confidence intervals) P = <0.001. SMR for vascular disease = 13.8 (7.2–36.5) P = <0.001. For persistent disease (n=6 pts) SMR = 16 (6.7–38.4) vs remission (n=54) SMR = 3.3 (1.7–6.7); after adjustment for age and sex relative risk of death for persistent disease was 10.7 (2.3–48.6) P = 0.002. Hypertension and diabetes mellitus were associated with significantly worse survival. Using a random effects model meta-analysis of seven studies (including our own) revealed an overall SMR of 2.2 (1.45–3.41) P = <0.001 indicating decreased survival. Pooled SMR for patients in remission (4 studies which compared the remission versus persistent disease) was 1.2 (0.45–3.2) P = NS and 5.5 (2.7–11.3) P = 0.001 for patients with persistent disease. Persistence of disease, older age at diagnosis, and presence of hypertension and diabetes appear as the main determinants of mortality.

Conclusions Overall mortality in CD is double that of the general population. Patients in remission fare much better than those with persistent hypercortisolism. SMR of 1.2 for patients in remission may not be statistically significant but could become so with larger numbers followed for a much longer time (30–40 years).

### P224

**Negotiating with a pea-sized hormone factory; the mediatry role of pituitary formyl peptide receptor (FPR) ligands in times of stress**

Vance Naughton, Bradley Spencer-Dene, Chris John & Julia Buckingham

1Imperial College London, London, UK; 2London Resaroch Institute, London, UK.

Pituitary folliculo-stellate cells express annexin-A1 (ANXA1), a mediator protein necessary for glucocorticoid (GC)-induced negative feedback of adrenocorticotrophic hormone (ACTH) release. ANXA1 acts via formyl peptide receptor (FPR) in man. Fpr1 in rodents. Whilst pituitary tissue does not express Fpr1, functional data suggested Fpr2/Fpr3-selective ligands mediate feedback-inhibition of ACTH.

**Hypothesis**

Fpr2-selective ligands inhibit secretagogue-induced ACTH release from pituitary tissue in vitro.

**Methods**

Firstly, we cultured anterior pituitary cells from Sprague–Dawley rats (n=12, male, 200–220 g) in CRH-stimulated (100 mmol/l) or basal conditions, along with peptide agonists for Fpr2 (WKYMVm (10−3, 10−4, 10−5 mol/l) and F2L (50, 100, 200, 400, 800 mmol/l)) or Fpr1 (IMLF (10−5, 10−4, 10−3 mol/l)). ACTH was measured by RIA after 4 h and data (mean ± S.E.M.) analysed by two-way ANOVA. Secondly, the pituitary Fpr2/3 distribution was determined by immuno-fluorescent detection of pituitary cell subtypes and green fluorescent protein (GFP) in sections of Fpr2/3-null/GFP-positive mice (n=4, male).

**Results**

Concordant with previous data, low dose (MLF did not affect ACTH release, confirming Fpr1 is not instrumental in ACTH regulation. Surprisingly, in separate experiments, two-way ANOVA revealed a significant stimulatory effect of WKYMVm and F2L on ACTH release (F(7,176)=71.38 and 49.17 respectively; P<0.0001). ACTH release from CRH-stimulated, WKYMVm-treated (10−3 mol/l) and CRH-stimulated, F2L-treated cells (800 mmol/l) versus CRH control was 29% (1680 pg/ml ± 111 vs 1320 pg/ml ± 75) and 60% (2953 pg/ml ± 188 vs 1817 pg/ml ± 227) higher, respectively (P<0.05). GFP as a proxy for Fpr2/3, was evident in corticotropes, somatotropes, and lactotropes, but, importantly, not in folliculo-stellates (anti-S100b).

**Conclusion**

Our work shows rodent Fpr2-selective ligands stimulate ACTH release and suggests Fpr2 does not mediate feedback-like regulation of pituitary ACTH release. Ongoing work will determine whether inhibition of ACTH is mediated by Fpr3 ligands, namely, 15-epi lipoxin A4.

### P225

**Pituitary dysfunction is uncommon in pituitary incidentalomas**

Anmara Tarik & Steven Peacey

Department of Diabetes and Endocrinology, Bradford Teaching Hospitals NHS Foundation Trust, Bradford, West Yorkshire, UK.

The frequent use of brain and head MRI/CT has lead to an increase in the number of incidental pituitary lesions reported, so called pituitary ‘incidentalomas’. Such lesions require endocrine evaluation of pituitary function and radiological follow up. We performed a retrospective study to examine the source of these referrals, size of the incidentalomas, frequency of pituitary dysfunction and changes during follow-up. We performed a search of the departmental endocrine database and clinical letters for the term ‘incidentaloma’ from 2005 onwards.

Thirty six patients, age ranged 23–86 years, 22 M14 were identified with pituitary incidentalomas. The majority of referrals were from the departments of neurology and ophthalmology, to a lesser extent from general practitioners, elderly care and psychiatry. Of these 36 pituitary incidentalomas, there were 18 macroadennomas, 14 microadenomas, 3 pituitary ‘hyperplasia’ and 1 intrasellar menngioma.

Following endocrine evaluation, hypopituitarism was rare and found in only one macroadennoma and not in any microadenomas. Hypersecretion was also infrequent; 1/18 macroadenomas secreting prolactin. Three microadenomas were secreting excess hormones; 2/14 prolactin, 1/14 growth hormone. The optic chiasma was radiologically involved in 7/18 macroadenomas; however visual fields were affected in only 2/18. Of those patients who did not undergo surgery (n=22), radiological follow up in the remainder, confirmed an increase in tumour size in 2/10 macroadenomas and 1/12 microadenomas.

We conclude that pituitary dysfunction is uncommon in pituitary incidentalomas, which may explain why these lesions are found ‘incidentally’ even though the majority of these are macroadenomas.

### P226

**A case of lymphocytic hypophysitis with spontaneous remission**

Anastasia Giotopoulou, Julie Kyaw Tun & Emma Ward

St James’ University Hospital, Leeds, UK.

A 19 year old girl presented at 39 weeks gestation with headache and blurring of vision. MRI showed an enhancing pituitary mass measuring 1.2 cm maximum diameter.
height, extending suprasellarly and distorting the optic chiasm. Visual field testing showed bilateral constriction. 0900 h cortisol was 502 nmol/l, thought to be relatively low for the stage of pregnancy and FT4 was 9.3 pmol/l suggesting TSH deficiency. Prolactin was appropriately raised for the gestational age at 495 mU/l.

A diagnosis of probable lymphocytic hypophysitis was made. Given her advanced gestational age, pituitary surgery or biopsy was not thought to be appropriate. Regular hydrocortisone was started with high dose cover during labour. She was also given 1mg of cabergoline, based on the presumption that dopamine agonist treatment would stop the lactotroph hyperplasia and result in some tumour shrinkage. She delivered a healthy baby girl a week later and was asked not to attempt to lactate.

A month later repeat MRI showed regression of the pituitary mass to 8 mm and visual fields showed a substantial improvement. Prolactin was 411 mIU/l and FT4 was 12.9 pmol/l, implying recovery of the pituitary-thyroid axis. The glucagon stimulation test showed a suboptimal peak cortisol at 429 nmol/l.

Consecutive MRI scans have shown a progressive reduction in the tumour size over subsequent months. Insulin stress test 7 months post delivery showed a peak cortisol of 498 nmol/l and a peak GH of 1.8 μg/l. Gonadotrophins are normal and she is menstruating regularly. She takes hydrocortisone for intercurrent illness and remains asymptomatic from her GH deficiency.

Lymphocytic hypophysitis is a rare condition, thought to be autoimmune in origin, with a strong predilection for women in the peripartum period. It usually presents with enlarging pituitary mass and variable degrees of hypopituitarism. This case illustrates that conservative management of lymphocytic hypophysitis should be considered, as in some cases it may remit spontaneously.

**Result**

The major indication for ITT was non-functioning pituitary macro adenomas. 76% of the cohort was hypoglycaemic (<2.0 mmol/l) for 60 min or more. The nadir plasma glucose (NBG) ranged from 0.1–4.6 mmol/l and correlated significantly with basal plasma glucose (BPG) (r = 0.56; P < 0.001), Insulin dose (r = 0.27; P < 0.001) and weight (r = 0.21; P < 0.01). 24 patients received an insulin dose exceeding 0.15 IU/kg body weight. These patients were characterized by higher weight (mean 93 vs 86 kg) and BPG (mean 5.7 vs 4.8 mmol/l) compared with the rest of the population.

Using multiple regression analysis, the factors determining nadir blood glucose were plasma glucose (b = 0.56; P < 0.001 20% contribution) and weight (b = 0.14; P < 0.05 2% contribution).

Only one patient had an adverse event during the test. He developed unstable angina and needed coronary artery by-pass surgery following a finding of 3 vessel coronary artery disease. The mean NPG and insulin dose in this patient was comparable with the rest of the population.

**Discussion**

The limitations of the ITT include the perceived risks of prolonged hypoglycaemia. Our data shows that it is a relatively safe test but that hypoglycaemia can be prolonged and unpredictable in a significant proportion of patients. We have also shown that baseline plasma glucose and the patient’s weight predict the nadir plasma glucose.

We therefore propose that a better way to avoid unnecessarily prolonged hypoglycaemia is the use of an insulin and glucose infusion with bed-side plasma glucose analysis.

---

**P227**

An unusual cause of a sellar mass presenting as a pituitary adenoma

Abdul Mohammed, Peshraw Amin & Carolyn Chee

Derby Hospitals NHS Foundation Trust, Derby, UK.

A 57-year-old man presented with a 6-month history of worsening headaches and bitemporal hemianopia on visual field examination. MRI of the brain revealed a 3 cm pituitary lesion impinging the optic chiasm and indenting the pons. Appearances of the lesion on imaging and the patient’s normal anterior pituitary hormonal profile were highly suggestive of a non-functioning pituitary adenoma and this was the presumed diagnosis. The patient underwent trans-sphenoidal resection of the tumour. Histopathology and immunohistochemical staining confirmed the unexpected diagnosis of a chordoma. Chordomas are rare malignant tumours of the axial skeleton arising from primitive remnants of the notochord. It can mimic a pituitary adenoma or craniopharyngioma. Tumours are usually slow-growing but can be locally aggressive and have high recurrence rates. Few literature reports have described pseudoprolactinomas, chordomas masquerading as prolactin-secreting tumours that may present a pre-operative diagnostic dilemma. Treatment options include extensive surgical resection, radiotherapy or both. This case demonstrates that although extremely rare, chordomas can directly involve the sellar region and mimic features of a pituitary adenoma. Clinicians should consider this as a differential diagnosis of a pituitary sellar or suprasellar mass.

---

**P228**

An audit of 220 insulin tolerance tests

Olubukola Ajala & Daniel Flanagan

Plymouth Hospitals NHS trust, Plymouth, UK.

Objective

We reviewed the depth and length of hypoglycaemia in a cohort of patients undergoing Insulin Tolerance Tests (ITTs). We evaluated the safety of the test, its reproducibility and explored factors that might predict the optimal dose of insulin.

Methodology

ITTs were performed at a teaching hospital in the south of England between 2005 and 2010.

Result

The major indication for ITT was non-functioning pituitary macro adenomas. 76% of the cohort was hypoglycaemic (<2.0 mmol/l) for 60 min or more. The nadir plasma glucose (NBG) ranged from 0.1–4.6 mmol/l and correlated significantly with basal plasma glucose (BPG) (r = 0.56; P < 0.001), Insulin dose (r = 0.27; P < 0.001) and weight (r = 0.21; P < 0.01). 24 patients received an insulin dose exceeding 0.15 IU/kg body weight. These patients were characterized by higher weight (mean 93 vs 86 kg) and BPG (mean 5.7 vs 4.8 mmol/l) compared with the rest of the population.

Using multiple regression analysis, the factors determining nadir blood glucose were plasma glucose (b = 0.56; P < 0.001 20% contribution) and weight (b = 0.14; P < 0.05 2% contribution).

Only one patient had an adverse event during the test. He developed unstable angina and needed coronary artery by-pass surgery following a finding of 3 vessel coronary artery disease. The mean NPG and insulin dose in this patient was comparable with the rest of the population.

Discussion

The limitations of the ITT include the perceived risks of prolonged hypoglycaemia. Our data shows that it is a relatively safe test but that hypoglycaemia can be prolonged and unpredictable in a significant proportion of patients. We have also shown that baseline plasma glucose and the patient’s weight predict the nadir plasma glucose.

We therefore propose that a better way to avoid unnecessarily prolonged hypoglycaemia is the use of an insulin and glucose infusion with bed-side plasma glucose analysis.

---

**P229**

20 year experience in the surgical management of Cushing’s disease in a UK tertiary referral centre

Zaki Hassan-Smith1, Alan Johnson2, Andrew Toogood1, Wiebke Arlt1, Mark Sherlock1 & Paul Stewart1

1University of Birmingham, Birmingham, UK; 2University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK.

Objective

The past 2 decades have seen advances in the surgical management of Cushing’s disease (CD). Our aim was to meet the need for current data on clinical features, long-term outcomes, and prognostic indicators.

Patients and methods

We conducted a retrospective study of 71 patients treated by trans-sphenoidal surgery (TSS) for CD. All patients were operated on by the same surgeon in a single centre between 1988–2009. Diagnosis was confirmed using Low and high dose DSTs, and CRH tests, with selected patients undergoing IPSS. 58 patients underwent microscopic-TSS at first surgery, whereas 13 had Endoscopic-TSS.

Results

Median follow up was 50 months (IQR 22–115 months). Median age at diagnosis was 39 years (IQR 30–50 years). Male:female ratio was 1:3.4. Follow up data were available on 67/71 patients. 82% (55/67) achieved initial clinical remission, of which 8 suffered disease recurrence. Mean time to recurrence was 3.9 years (IQR 3 months–14 years). 3 outcome groups were identified: ‘Long-term Cure’ 70% (47/67), ‘Persistant Disease’ 18% (12/67), and ‘Recurrent Disease’ 12% (8/67).

Long-term cure rates were higher in patients with undetectable (<30 nmol/l) post-operative cortisol levels (87%). They were also increased in patients with histology positive for ACTH-containing adenomas (82%), compared to those with negative histology (57%). Further treatment for patients with recurrent/persistent disease included revision TSS (n = 11), Radiotherapy (n = 7), and Adrenalectomy (n = 7). Common complications following TSS were transient DI (60%), and CSF leak (16%). Hypopituitarism was present in 81% of patients at final follow up. Mortality was increased in persistent/recurrent disease, compared to the long-term cured (P = 0.04). 4 deaths were documented (2 recurrent, 1 persistent disease, 1 cured 20 years previously).

Conclusion

Our favourable remission/cure rates, serve to underline the importance of an experienced surgeon in the management of CD. Favourable prognostic factors include undetectable post-operative cortisol and ACTH-positive histology. The reduced mortality in the long-term cured, demonstrates the importance of aggressive treatment of CD.
**P230**

**Suspected spontaneous resolution of pituitary cushing’s disease**

Wen Bun Leong & Darren Warner

Princess Royal Hospital, Telford, UK.

A 49-year-old lady was referred by her GP with lethargy, oligomenorrhea and persistent hypertension despite three anti-hypertensives (lisinopril, amlodipine, and atenolol). She denied exogenous steroid usage and had no other medical history.

On examination, she had features of Cushing’s syndrome including plethora, moon face, central obesity and androgenisation of her facial skin. She weighed 98 kg.

Overnight (1 mg) low dose dexamethasone suppression test disclosed no suppression of cortisol. High dose dexamethasone suppression test suggested pituitary-dependant Cushing’s. A confirmatory CRF test showed marked rise in serum cortisol and ACTH. MRI brain showed a 9 mm low signal area in the left side of pituitary post gadolinium in keeping with pituitary adenoma. Her pituitary profile was otherwise unremarkable.

She was referred for hypophysectomy. Four months later, on the day of surgery, her pre-operative MRI brain showed spontaneous shrinkage of her pituitary adenoma with no signs of haemorrhage to suggest apoplexy. Her surgery was cancelled.

Two months later, she had lost 8 kg in weight with a normalising body habitus and no proximal myopathy. Her repeat low dose dexamethasone suppression test was normal; CRF testing shows residual but much improved cortisol secretion 10 months later. Her static pituitary screen is otherwise normal and her weight loss continues. Repeat MRI pituitary showed a normal pituitary gland in August 2010.

This is the first case report which showed a near spontaneous resolution of the pituitary Cushing’s disease without any medical or surgical intervention. Possible causes include spontaneous infarction of the tumour.

**P231**

**Tri-phasic changes in sodium levels post pituitary surgery**

Hollie Ellis1, David Webb1, Iain Robertson2, Trevor Howlett1 & Miles Levy3

1Department of Endocrinology, Leicester Royal Infirmary, Leicester, UK; 2Department of Neurosurgery, Queens Medical Centre, Nottingham, UK.

Case

A 73-year-old lady presented with hypernatraemia six days post-transphenoidal surgery for a non-functioning pituitary macro-adenoma. Peri-operatively she developed diabetes insipidus requiring short term desmopressin whilst on the neurosurgical ward. At post-operative presentation she complained of weakness, confusion and nausea; sodium 125 mmol/l, serum osmolality 268 mOsmol/kg, urine osmolality 474 mOsmol/kg. Over the next few days she became symptomatically worse and her hypernatraemia more severe; sodium 114 mmol/l, serum osmolality 252 mOsmol/kg, urine sodium 52 mmol/l.

A diagnosis of post-operative SIADH was made and she was admitted for optimisation of fluid balance. Because she was clinically dehydrated, she was given slow intravenous normal saline. After 5 days her sodium gradually increased to 140 mmol/l and she was symptomatically much improved. Nine days later, her sodium increased to 145 mmol/l, serum osmolality 311 mOsmol/kg, urine osmolality 464 mOsmol/kg. Subsequently her sodium rose to a peak of 150 mmol/l; she developed polyuria and polydipsia and also reported numbness in the face and both feet and general loss of balance. A diagnosis of diabetes insipidus was made on the basis of the high urine osmolality and polyuria, and the patient was started on DDAVP. We have not yet explained her neurological symptoms and she awaits a post-operative MRI brain and pituitary fossa.

Discussion

This case is interesting as it displays the tri-phasic response pattern of sodium that can be seen post pituitary surgery. The development of SIADH is thought to be due to degeneration of paraventricular neurones, which may predispose to permanent diabetes insipidus. This case highlights the need to closely observe sodium levels in the short to mid-term post pituitary surgery. We discuss a possible short term role for the vaptans in temporary post operative SIADH which might prevent hospital admission in this situation.

**P232**

**Rare case of disseminated adenocarcinoma prostate metastasising to the pituitary, with a normal PSA**

Huong Trinh1, Mohsen Javadpour2, Atik Baborie1, David Ewins1, Niru Goenka1 & Franklin Joseph1

1Department of Diabetes and Endocrinology, Countess of Chester Hospital NHS Foundation Trust, Cheshire, UK; 2Department of Neurosurgery, Walton Centre for Neurology and Neurosurgery, The Walton Centre NHS Foundation Trust, Liverpool, UK; 3Department of Histopathology, Walton Centre for Neurology and Neurosurgery, The Walton Centre NHS Foundation Trust, Liverpool, UK.

Mr DP was referred by palliative care to ophthalmology with anisocoria, diplopia and nystagmus. He was known to have a poorly differentiated carcinoma of the prostate with skeletal metastasis (Gleason score 4+5=9). He was on hormonal therapy and had received radiotherapy to the cervical spine. PSA was normal, at 5.31 μg/l.

An MRI scan revealed a 12 mm mass in the left side of the pituitary gland, displacing the stalk to the right and lying close to the inferior surface of the optic chiasm. Prostatectom was elevated at 897 μg/l in keeping with stalk compression. IGF1 was normal at 24 μg/l and cortisol peaked at 626 nmol/l on a short synacthen test. A repeat MRI 3 months later showed the tumour had grown to 15.5 mm, and was extending into the cistern touching the chiasm from below. Although a non-functioning pituitary adenoma was more probable in this location, the rate of growth was suggestive of possible metastasis.

Image guided endoscopic trans-sphenoidal resection of the tumour was arranged, and histological examination confirmed a moderately differentiated adenocarcinoma consistent with a metastatic deposit of the prostate. There were no postoperative visual complications. Follow up imaging at 3 months showed tumour residue reduced to 7 mm. Postoperative radiotherapy was not initiated as the patient became too unwell due to disseminated malignancy.

Prostate cancer is usually associated with hepatic, skeletal and pulmonary metastases. Intracranial metastases are uncommon, with involvement of the pituitary gland rarer still. Small cell carcinoma of the prostate has a greater predilection to distant metastasis but this case represents a typical adenocarcinoma. Although unusual, this case highlights the need to consider prostate metastasis to the pituitary, even with a normal PSA, as a metastatic deposit would potentially be more aggressive than a concurrent pituitary adenoma and would warrant earlier intervention.

**P233**

**An unusual case of extremely high prolactin due to stalk disconnection hyperprolactinaemia**

Haliza Hannif1, Paul V Marks2, Azzam Ismaili2 & Robert S Moissey1

1Huddersfield Royal Infirmary, Huddersfield, UK; 2Leeds Teaching Hospitals NHS Trust, Leeds, UK.

A 75-year-old man was admitted acutely with a 1 week history of headaches, reduced visual acuity, diplopia and ptosis of his left eye. Examination confirmed a left III and VI nerve palsies with decreased visual acuity in the left eye. A CT and subsequent MRI revealed pituitary mass lesion measuring 2.4 x 2.1 x 1.5 cm with extrusion into the left cavernous sinus. The pituitary stalk appeared thickened and was deviated to the right. The optic apparatus was uninvolved.

He had a background history of adenocarcinoma of the ascending colon with right hemicolectomy in March 2007 and in July 2009 he had undergone a left upper lobe metastasectomy for a lung metastasis. A repeat CT of his chest and abdomen revealed a left upper lobe metastas with enlarged mediastinal lymph nodes.

His pituitary hormone profile showed a markedly elevated prolactin and anterior pituitary failure: prolactin 8766 mU/l (50–700), LH 0.6 U/l (1.0–9.0), FSH 2.0 U/l (1.0–10.0), testosterone <0.4 nmol/l (3.0–27.0), IGF1 8.0 nmol/l (7.0–22.0), cortisol 68 nmol/l (184–623), TSH 0.08 mU/l (0.2–4.0) and T4 7.9 pmol/l (8.0–21.0). Macroprolactin was excluded. He was commenced on cabela mine 500 μg once daily, hydrocortisone and levethyroxine. His prolactin corrected rapidly, falling to 83 nmol/l after two weeks. However a repeat MRI showed no change in the size of the pituitary mass. Subsequently he underwent transphenoidal surgery to debulk his pituitary mass and confirm the aetiology.

Histology showed moderately differentiated adenocarcinoma compatible with a metastasis from gut primary. Immunohistochemistry showed no evidence of prolactin secretion from within the tumour. He deteriorated quickly and died four months later.

This case demonstrates an unusually high prolactin level which is not due to a prolactinoma but instead is due to stalk disconnection hyperprolactinaemia from a rare occurrence of a pituitary metastasis.
P234
The risk of cardiac valvulopathy in cabergoline-treated endocrine patients in a district general hospital
Saravanaan Balaguruswamy, Natalie Lewis, Sid McNulty & Niall Furlong
St Helens and Knowsley NHS Trust, Merseyside, UK.

Aim
Over the last decade, cardiac valvular fibrosis has been associated with the use of high dose (ergot-derived) dopamine agonist therapy in Parkinson’s disease. Although the risk in endocrine patients appears significantly lower, routine echocardiography monitoring is now recommended. This study evaluated the incidence of significant cardiac valvulopathy in endocrine patients treated with cabergoline in a District General Hospital Population, and compliance with MHRA 2008 recommendations for chronic endocrine use of ergot-derived dopamine agonists.

Methods
We identified 18 patients treated with cabergoline in the Endocrine Clinic over a 6 year period (August 2004 – May 2010), 16 for hyperprolactinaemia (8 with microadenoma, 4 with macroadenoma and 4 with primary hyperprolactinaemia) and 2 for acromegaly. For hyperprolactinaemia mean (± s.d.) age was 40.75 (± 17.79) years, 88% were female (n = 16). The median (range) cumulative dose of cabergoline was 88 mg (9–781) and treatment duration 33 months (6–152). Both acromegaly patients were female, age 81 and 73 years, cumulative cabergoline doses 1564 and 715 mg, and treatment duration 10 years and 6 years, respectively.

Results
Baseline echocardiography was documented in 86% patients. 81% of eligible patients received echocardiography monitoring as per MHRA guidelines. In the hyperprolactinaemia patients, no significant cardiac valvulopathy was identified (one case of mild aortic regurgitation (with no serial progression), all others echocardiographically normal). Both acromegaly patients were found to have significant aortic valve disease (moderate AR).

Conclusions
In our cohort we found no evidence of new or worsening valvular lesions in hyperprolactinaemic patients treated with cabergoline. In line with other similar studies, the risk of cardiac valvulopathy in hyperprolactinaemic patients treated with standard doses of cabergoline appears low. Although acromegaly is associated with cardiac valvulopathy per se, it is noteworthy that these patients received higher doses and longer duration of treatment and both developed valvulopathy.

P235
Cancer stem cells with a stabilising mutation in beta-catenin are implicated in the aetiology of human adamantinomatous craniofibroma
Cynthia Lilian Andoniadou1, Carles Gaston-Massuet1, Paul Le Tissier 2, Lorna Smith1, Elizabeth Davies1, Annalisa Gastaldello1, Iain Thompson1, Saravanan Balaguruswamy, Natalie Lewis, Sid McNulty & Niall Furlong 1,2
1Department of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK; 2Department of Endocrinology, Athens Polyclinic Hospital, Athens, Greece.

Cushing’s disease (CD) is a rare condition, associated with significant morbidities. The long-term morbidities in a series of patients with CD who presented in two tertiary referral centres between 01/1967-06/2009 were assessed. All information was collected as documented in the records of the patients.

224 patients were identified (174 females) with median age at diagnosis 39 years (range 10–76; females 38 (12–72) – males 40.5 (10–76)) and median follow-up 113 months (0–550) (mean 143 months (± 121)). Treatment modalities were: TSA 144 patients (cured 70%), TSA + external radiotherapy 22 (cured 32%), TSA + bilateral adrenalectomy 21 (cured 100%), TSA + bilateral adrenalectomy + radiotherapy 8 (cured 100%), bilateral adrenalectomy 19 (cured 100%), radiotherapy 3 (cured 33%). Two patients are waiting for surgery, 4 had been treated medically due to high surgical risk, and one died before any treatment. The median time between cure and last assessment was 102 months (0–549) (data available for 192 patients (85.7%)). At last assessment, 70.5% of the subjects were considered cured (157/224). Morbidity rates and median (range) age at last follow-up were: hypertension 35.3% (79.8% cured – 54 years (17–82)) dyslipidaemia 22.2% (75.4% cured – 52 years (25–82)); DM2 13.4% (86.6% cured – 55 years (18–75)); depression 14.3% (81.3% cured – 60 years (22–78)); osteopaenia/ostoporosis 39.7% (76.4% cured – 52 years (18–78)); cardiovascular disease 9.4% (66.6% cured – 52 years (26–71)); cerebrovascular disease 3.6% (62.5% cured – 59 years (26–71)); kidney stones and/or dysfunction 53.5% (83.3% cured – 48 years (30–69)); osteopenia 0.4% (100% cured (39–43)).

High proportions of various morbidities in CD rectify themselves after successful -treatment but significant morbidities also remain, particularly with regard to hypertension, dyslipidaemia, osteopaenia/ostoporosis and are likely to have an impact on long-term mortality. Whether these morbidities are the result of previous steroid exposure or of possible hypopituitarism remains to be clarified.

P236
Long term morbidities in a large series of patients with Cushing’s disease
Georgia Ntali1,2, Thomas Siamatras1, John Komninos1, Stelios Tsagarakis1,2, John Wass2 & Niki Karavitaki1
1Department of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK; 2Department of Endocrinology, Athens Polyclinic Hospital, Athens, Greece.

Cushing’s disease (CD) is a rare condition, associated with significant morbidities. The long-term morbidities in a series of patients with CD who presented in two tertiary referral centres between 01/1967-06/2009 were assessed. All information was collected as documented in the records of the patients. 224 patients were identified (174 females) with median age at diagnosis 39 years (range 10–76; females 38 (12–72) – males 40.5 (10–76)) and median follow-up 113 months (0–550) (mean 143 months (± 121)). Treatment modalities were: TSA 144 patients (cured 70%), TSA + external radiotherapy 22 (cured 32%), TSA + bilateral adrenalectomy 21 (cured 100%), TSA + bilateral adrenalectomy + radiotherapy 8 (cured 100%), bilateral adrenalectomy 19 (cured 100%), radiotherapy 3 (cured 33%). Two patients are waiting for surgery, 4 had been treated medically due to high surgical risk, and one died before any treatment. The median time between cure and last assessment was 102 months (0–549) (data available for 192 patients (85.7%)). At last assessment, 70.5% of the subjects were considered cured (157/224). Morbidity rates and median (range) age at last follow-up were: hypertension 35.3% (79.8% cured – 54 years (17–82)) dyslipidaemia 22.2% (75.4% cured – 52 years (25–82)); DM2 13.4% (86.6% cured – 55 years (18–75)); depression 14.3% (81.3% cured – 60 years (22–78)); osteopaenia/ostoporosis 39.7% (76.4% cured – 52 years (18–78)); cardiovascular disease 9.4% (66.6% cured – 52 years (26–71)); cerebrovascular disease 3.6% (62.5% cured – 59 years (26–71)); kidney stones and/or dysfunction 53.5% (83.3% cured – 48 years (30–69)); osteopenia 0.4% (100% cured (39–43)).

High proportions of various morbidities in CD rectify themselves after successful treatment but significant morbidities also remain, particularly with regard to hypertension, dyslipidaemia, osteopaenia/ostoporosis and are likely to have an impact on long-term mortality. Whether these morbidities are the result of previous steroid exposure or of possible hypopituitarism remains to be clarified.

P237
Molecular and functional characterisation of an endocannabinoid system in LjT2 gonadotrophs
Lorna Smith1, Elizabeth Davies1, Annalisa Gastaldello1, Iain Thompson1, Edward Perello1, Marta Konobius2, Michelangelo Campanella1 & Robert Fowkes1
1Royal Veterinary College, London, UK; 2Barts and the London School of Medicine and Dentistry, London, UK.

Cannabinoids are known to exert effects throughout the endocrine system, including in the anterior pituitary. Whilst cannabinoid receptors (CB1 in particular) are expressed in numerous tissues, expression and function of an endocannabinoid system within specific anterior cell types has not been investigated. We have used well-characterised gonadotroph (LjT2 and aT3-1) and somatolactotroph (GH3) cell lines to establish the expression and functional role for cannabinoid signalling. Initial RT-PCR studies established that LjT2 cells expressed Crn1, as well as the cannabinoid synthetic enzymes, Faah, Mgly and Napepld. aT3-1 and GH3 cells, and primary mouse pituitary tissue only expressed some of these components. Having established that LjT2 cells expressed a complete endocannabinoid system, functional studies were undertaken. As the CB1 (Crn1) receptor couple to Gaαi3, in many tissues, the potential inhibitory effect of CB1 signalling was determined in LjT2 cells. Following transient transfection with reporter genes expressing either the human αGSU promoter, the murine Egr-1 promoter, or a Gal4CREB two-hybrid system, cells were stimulated with 0 or 10 μM Forskolin (FSK, adenylyl cyclase activator), in the absence or presence of a range of concentrations of HU210 (a CB1 agonist). HU210 caused an inhibition of the αGSU and Gal4CREB response to FSK, but failed to alter Egr-1 promoter activity. Similar studies in GH3 cells revealed dramatic inhibition in FSK-stimulated αGSU promoter activity in the presence of HU210, suggesting that CB1 couples to Gaαi3 in both LjT2 and GH3 cells. As CB1 receptors can couple to numerous G-protein α-subunits, dynamic confocal imaging of intracellular calcium concentrations ([Ca2+]i) was performed, following Fluo4AM loading. HU210 caused a rapid increase in [Ca2+]i in LjT2 cells, suggesting potential coupling to Gaαq11. Collectively, these data reveal gonadotrophs and somatolactotroph lineage cells to be putative targets of endocannabinoid signalling in the pituitary, which may reveal potential therapeutic benefits for fertility and growth disorders.
P238
Assessment of the feasibility of early hospital discharge following trans-sphenoidal pituitary surgery
Sujata Biswas1, Thomas Barber1, Simon Cudlip2 & John Wass3
1Department of Endocrinology, OXDEM, John Radcliffe Hospital, Oxford, UK; 2Department of Neurosurgery, John Radcliffe Hospital, Oxford, UK.

Objectives
Reducing length of inpatient stay following trans-sphenoidal pituitary adenectomy (TSA) could create significant financial saving for the NHS. We assessed early complication rates post-TSA to determine feasibility of early hospital discharge (3 days) post-TSA.

Methods
We identified retrospectively 60 patients who underwent TSA at the John Radcliffe Hospital. These consisted of patients with a pre-operative confirmed diagnosis of non-functioning adenoma (NFA, n = 20), Cushing’s disease (n = 20) and acromegaly (n = 20). Data collected included age, gender, town of residence, length of stay, requirement for hydrocortisone and desmopressin at discharge, serum [Na] excursions and post-operative complications. Data are reported as mean (s.d.). For comparison between sub-groups, the ANOVA test was used.

Results
The NFA sub-group was older than other sub-groups (NFA, 60.9 years (14.3); Cushing’s, 44.4 years (13.6); acromegaly, 43.7 years (13.4); P < 0.0001). There were 30 male and 30 female patients, and 42 patients lived outside Oxfordshire. Average length of in-patient stay was significantly greater for the Cushing’s sub-group compared to others (NFA, 6.0 days (5.6); Cushing’s, 12.4 days (11.9); acromegaly, 5.6 days (1.8); P = 0.01). Discharge on hydrocortisone therapy was required for NFA (n = 15), Cushing’s (n = 18) and acromegaly (n = 8); P = 0.002. Discharge on desmopressin was required for NFA (n = 3), Cushing’s (n = 6) and acromegaly (n = 2); P = NS. Serum [Na] excursions in the early post-operative period were equivalent between subgroups (NFA, 2.5 mmol/l (3.5); Cushing’s, 3.5 mmol/l (3.0); acromegaly, 3.5 mmol/l (3.5); P = NS). Early post-operative complications (including CSF leak, epistaxis, chest infection and need for further surgery) occurred significantly more frequently in the Cushing’s sub-group (NFA, n = 3; Cushing’s, n = 11; acromegaly, n = 5; P = 0.01).

Conclusions
Our data clearly demonstrate that Cushing’s patients are significantly more likely to develop early post-operative complications and require longer hospital stay compared with acromegaly and NFA patients. Although our data do not support a policy of early discharge post-TSA in Cushing’s patients, they do support an audited trial of early post-TSA discharge in patients with NFA and acromegaly.

P239
A debilitating case of cushing’s disease?
Anjali Amin, Emma Hatfield & Karim Meeran
Imperial College Healthcare NHS Trust, London, UK.

A 48-year-old lady presented to neurology with a 2 year history of progressive leg weakness, rendering her wheelchair-bound. Neurological examination revealed a proximal myopathy. She had a 2 year history of diabetes, and an endocrine disease was considered, however the severity of weakness led to further investigation. She went on to have inferior petrosal sinus sampling, which supported a diagnosis of possible motor neurone disease (MND).

MRI scan of the pituitary showed a bulky gland, asymmetry of the fossa and stalk deviation. She went on to have inferior petrosal sinus sampling, with a baseline and stimulated ACTH to peripheral ratio consistent with a pituitary source of ACTH.

A diagnosis of a proximal myopathy secondary to ACTH-dependent Cushing’s disease was considered, however the severity of weakness led to further investigation. Nerve conduction studies were performed, which supported a diagnosis of possible motor neurone disease (MND).

P240
Cranial nerve palsy related to previous radiotherapy in a treated case of acromegaly
Allison Martin & Gul Bano
St Georges Hospital, London, UK.

A 56-year-old woman presented with an acute onset of left sided ptosis, diplopia and failure of upward gaze almost 20 years after conventional pituitary irradiation for a growth hormone secreting tumour. Her visual fields were full. Visual acuities were 6/9 in the right eye and 6/6 in the left eye. She had a complete left third nerve palsy. She was growth hormone deficient and had primary hypothyroidism, hypercholesterolemia and hypertension. These were well controlled on treatment. A MRI of the pituitary showed a haernorrhagic cyst. Her pituitary MRI had been stable for many years. There are only two other reported cases of radiation induced acute third nerve palsy in the literature. This condition is rare but has lasting debilitating effects. Conventional radiotherapy is a highly effective form of treatment for pituitary tumours. It is recommended as second or third line treatment after subtotal resection of large aggressive tumours to prevent tumour regrowth; after near total resection of hormone secreting adenomas which demonstrate hormone activity postoperatively and also after a pituitary apoplectic event to reduce the risk of recurrence. It is sometimes used as first line treatment in patients deemed unfit for surgery. Hypopituitarism is still the most common long term side effect of pituitary irradiation with an incidence of 13-56%. If single doses of 2.0 Gy and total doses of 50 Gy are not exceeded the risk of other complications is small: brain necrosis 0.2%, deterioration in vision 1.7%, vascular changes 6%, neuroendocrine disorders such as dementia 0.7% and secondary malignancies 0.8%.

P241
Worsening of thyroid functions following surgical removal of a combined GH/TSHoma
Nisha Kaimal, Ahmed Elsadig, Donal Bradley, Kanna Gnanalingham & Tara Kearney
Salford Royal hospital, Manchester, UK.

A 51 year old lady presented with a 3 week history of persistent headache, sweating, increase in shoe and ring size and proggynosis. Imaging confirmed an 18 × 19 mm pituitary macroadenoma indenting the optic chiasma. Visual fields were normal. Bloods: IGF1-181 nmol/l (11.3–30.9 nmol/l), prolactin 76 mU/l (102–496 mU/l), LH 17.2 IU/l, FSH 17.2 IU/l, oestradiol <70 pmol/l, prolactin 204 mU/l, IGF1 34.3 nmol/l, TSH 1.90 mU/l T4 18.3 pmol/l. She had 2 low-dose dexamethasone suppression tests, which showed failure to suppress cortisol:

<table>
<thead>
<tr>
<th>Cortisol (nmol/l)</th>
<th>ACTH (ng/l)</th>
<th>Cortisol (nmol/l)</th>
<th>ACTH (ng/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T = 0</td>
<td>417</td>
<td>59.4</td>
<td>557</td>
</tr>
<tr>
<td>T + 48 h</td>
<td>108</td>
<td>101</td>
<td></td>
</tr>
</tbody>
</table>

MRI of the pituitary showed a pituitary macroadenoma. She underwent successful transsphenoidal surgery with a day 4 cortisol of 67 nmol/l, and history confirming a corticosteroid adenoma. She was discharged into a rehabilitation programme on hydrocortisone, and 6 months later her weakness was still present, although her weight and acne had improved. This unusual case of cushing’s disease presenting concurrently with MND has not previously been described in the literature. It highlights the importance of looking for other pathologies, and not making the assumption that all the clinical features are due to the cushing’s disease.
Carbimazole dose titration brought the FT4 levels to normal range but the TSH continued to rise. This suggested the diagnosis of a combined GH/TSHoma. Repeat OGTT showed borderline failure of suppression of growth hormone but MRI was unchanged. Somatostatin analogues, SOM230, pituitary irradiation and thyroidecmy were discussed. A multi-disciplinary decision was made to commence him on octreotide prior to definitive radiotherapy. 

There are very few cases in literature of GH/TSHoma. This case highlights the challenges in the diagnosis and management of this relatively rare tumour. While growth hormone excess was the prominent clinical and biochemical feature pre-operatively, the thyroid axis abnormality was the predominant feature post-operatively, even before carbimazole was commenced. This case also illustrates the significant increase in TSH in response to a small reduction in FT4 levels – a known characteristic of this tumour.

**P242**

**Pitfalls in transphenoidal surgery: a 10 years experience**

Kanchan Mukherjee, Virender Khosa, Suresh Mathuriva, Anil Bhansali, Pinaki Dutta, Nilanjan Khandelwal, Paramjeet Singh, Rakesh Vasishtha, Bhisham Radotra, Ashis Pathak, Sunil Gupta, Manoj Tewari & Rajesh Chhabra

Postgraduate Institute of Medical Education and Research, Chandigarh, India.

Three hundred and thirty six cases of pituitary adenomas were operated by the transphenoidal route in the last 10 years by a single surgeon. Majority of these were giant non functioning adenomas. Prolactinomas were excluded unless a part of the dual adenoma.

In nearly 80% of tumours, total or near total removal was achieved. Long term outcome with follow up of more than 5 years revealed recurrence requiring re-surgery in only 4 out of 102 patients. Endocrine recovery was seen in many with permanent added hormonal deficit in around 5% of cases. Added visual deficit was seen in 1 patient.

Mortality was 14 patients and the avoidable reasons will be discussed. The advantages and disadvantages of an aggressive approach in a developing country were the cost of hormonal supplementation is prohibitive will be highlighted.

**P243**

**Pituitary apoplexy is a rare endocrine emergency, historically confused with other acute medical conditions, which delayed the diagnosis, however with advances in brain imaging, diagnosing this condition is much easier**

Acharya Jayashekar & Daniel Planagan

Derriford Hospital, Plymouth, UK.

We describe seven patients with pituitary apoplexy diagnosed between July 2009 and Oct 2010. 

Presentation and Management

Five patients presented with sudden onset headache, nausea and vomiting. One developed headache while on carbegoline and other presented with fluctuating level of consciousness.

All but two patients were inpatients at the hospital when diagnosed and all had MRI scan of pituitary to confirm the diagnosis and any mass effects.

All but two patients had visual field assessment. These two patients had cognitive impairment that made this test impossible.

Six patients had baseline pituitary function test. Four patients had insulin stress test to assess dynamic pituitary function. 

Three patients underwent microsurgery and open transphenoidal transsphenoidal hypophysectomy due to the presence of focal neurology (visual field defect and third cranial nerve palsy). Five patients were managed conservatively.

All seven patients were started on hydrocortisone after the diagnosis and two were able to come off hydrocortisone after dynamic testing.

All seven patients are under the follow up of Endocrinologist.

Summary and Conclusion

Our case series shows we are following the UK guidelines for the management of Pituitary apoplexy. MRI scan is very sensitive in diagnosing pituitary apoplexy even when there is low clinical suspicion.

**P244**

**BAC recombinerineering to understand the role of the alternative promoter in the regulation of prolactin expression**

Raheela Awaissen, Anne McNamara, Claire Harper, Anthony Anderson, Dave Spiller, Sabrina Sempri, John Mulkins, Julian Davis & Michael R L White

1 Institute of Integrative Biology, University of Liverpool, Liverpool, UK; 2Queen’s Medical Research Institute, University of Edinburgh, Edinburgh, UK; 3Faculty of Medical and Human Sciences, University of Manchester., Manchester, UK.

Alternative promoters control many genes including prolactin (PRL), which in man is expressed at extra-pituitary sites controlled by an alternative promoter located 5.8 Kbp upstream of the pituitary transcription start site. Previous studies using short promoter fragments may be misleading, as the human hPRL genomic locus has many conserved far-upstream regions. To study the function of the alternative promoter, we engineered a bacterial artificial chromosome (BAC) expressing Luciferease (Lac) under the control of a 163 Kbp hPRL genomic fragment (1), deleting the entire 5 kbp pituitary promoter by BAC recombinerineering, leaving intact the upstream exon 1a and alternative promoter. The alternative splice acceptor site was reintroduced after mutating three adjacent Pit-1 binding sites. This alternative PRL promoter BAC-Lac reporter gene (AP-BAC-Lac) was used to generate stably-transfected rat pituitary GH3 and Jurkat lymphoblastoid cell lines, representing cellular models of pituitary and extra-pituitary sites of expression.

Surprisingly, AP-BAC-Lac was active in both GH3 and Jurkat cell lines, with greater signal measured in pituitary cells. PRL-regulating stimuli, including FGFR2, forskolin and PMA, induced an increase in AP-BAC-Lac expression in the GH3 recombiant stable clones (similar fold-activation to that seen from the pituitary PRL promoter). Oestrogen and TNFα failed to induce AP-BAC-Lac expression, in contrast to their strong induction of PRL pituitary promoter activity in GH3 cells. Real-time luminescence imaging of living cells showed dynamic patterns of expression, as seen with pituitary promoter constructs, indicating that cyclical transcriptional function is shared by the alternative promoter. These results provide new insights into how alternative promoters differentially or coordinately regulate PRL expression. Our data indicate that both promoters share activity within the same cell, and both display cyclical function. In this context, the AP-BAC-Lac generated in this study offers a powerful model approach to understand mechanisms responsible for differential activation of alternative promoters in alternate sites of gene expression.

**P245**

**Evaluation of the interaction of phosphodiesterases 2A and 4A5 with the aryl hydrocarbon receptor interacting protein in pituitary cells**

Carole Lennox, Giampolo Trivellin & Marta Korbonits

Barts and the London School of Medicine and Dentistry, London, UK.

Background

Aryl hydrocarbon receptor interacting protein (AIP) mutations have been identified in ~15% of patients with familial isolated pituitary adenomas (FIPA). In addition, dysregulation of the cyclic adenosine monophosphate (cAMP) signalling pathway has been identified in both syndromic and sporadic somatotropinomas. While crosstalk between these two systems is known to occur, the exact mechanism of interaction remains elusive. The identification of direct binding between AIP and two cAMP-hydrolysing phosphodiesterases PDE2A and PDE4A5 may provide a starting point to explore this relationship (1, 2).

Aims and objectives

To investigate the presence of PDE2A and PDE4A4 in pituitary tumours. To assess whether overexpression of AIP, PDE4A5 and PDE2A affects their binding partner’s expression.

Methods

Presence of PDE2A and PDE4A4 in pituitary tumour cDNA samples was evaluated by RT-PCR. Rodent pituitary somatotroph cell line (GH3) were transiently transfected with wild type AIP, PDE2A, PDE4A5, mutant PDE4A5 and empty vector. Expression of AIP, PDE2A and PDE4A5 was assessed by RT-PCR and western blot.

Results

PDE2A and PDE4A4 was found to be present within pituitary adenomas. There was no significant difference in PDE2A and PDE4A4 expression between tumour subtypes and normal pituitary samples. There was no significant effect of AIP, PDE2A and PDE4A4 transfection on the cellular levels of their binding partners.
Discussion
AIP binding partners are present in sporadic pituitary adenomas but there was no difference in PDE2A and PDE4A4 expression in pituitary adenomas types or normal pituitary. Increased levels of AIP, PDE2A and PDE4A5 did not alter the expression of each other therefore different approaches are needed for the identification of possible tumorigenic mechanism involving these proteins.

His GST showed flat cortisol response with peak of 293, confirming cortisol deficiency and GH peak was only < 0.3 mU/l confirming GH deficiency. His MRI pituitary showed Empty sella with no evidence of intra sellar mass or Haemorrhage. visual fields were normal. He was commenced on Hydrocortisone replacement followed by Thyroxine and Testosterone replacement. His energy levels had improved and patient felt much better. Now he is under regular endocrine follow up.
Hypopituitarism is a well recognised and rare complication of bacterial meningitis and only few cases have been reported in the literature. Endocrinologist should be aware of this potential complication of meningitis.

P247
Hypopituitarism: is it due to bacterial meningitis?
Daniel Kannappan, Sami Kenz, Selena Farook & Georg Brabant
Christie Hospital, Manchester, UK.

A 75-year-old man presented to Emergency department with fever and 3 episodes of tonic clonic seizures. He had recent ear infection. He was intubated and ventilated and taken to ICU.
He had CT head which was normal. Lumbar puncture results were consistent with Pneumococcal meningitis. He was treated with appropriate antibiotics. During recovery he had persistent hyponatraemia. He was reviewed by Endocrine team and his TST, cortisol and other pituitary hormones were normal. His repeat CT head was normal. Further investigations confirmed SIADH possibly secondary to meningitis.
He presented to the GP 8 months later, with increasing tiredness and lethargy. Investigations showed testosterone was 0.4 nmol/l, FSH 2 U/l and LH 1 U/l. His TSH was 1.92 mU/l with free T4 7.5 pmol/l. His cortisol was 120 nmol/l and prolactin was 471 mU/l.
He was reviewed in the Endocrine clinic and Basal pituitary function test and Glucagon stimulation test were arranged.

Discussion
With the advent of vaptans as a therapeutic option, rapid and precise diagnosis of SIADH is essential. SIADH due to paraneoplastic stimulation of AVP is an expected but not the only cause of hyponatraemia in cancer patients. Therefore a precise evaluation of the cause of hyponatraemia is necessary before any therapeutic approach. Using Copeptin, a stable marker of AVP, which is stoichiometrically released with AVP, we evaluated 49 patients with hyponatraemia due to various forms of cancer. We followed classical diagnostic criteria for the assement of hyponatraemia which included clinical evaluation of the fluid status, measurement of serum cortisol, thyroid hormones, glucose, serum and urine osmolality and urinary sodium excretion. Only patients with hyponatraemia of <130 nmol/l were selected for the study. Serum copeptin levels were measured in addition to the above investigations with a specific immunosassay (detection limit of 1.7 pmol/l). The maximal inter and intra-assay CV being 6.5% for copeptin.
Compared to a control group and to the physiological variation found in the pattern of 24 h rhythm of copeptin obtained by sampling every 20 min in healthy subjects, 20 patients exceeded the maximal range of copeptin obtained in these healthy controls. The mean copeptin value of these patients (36 pmol/l) is more than twice the upper limit of normal of healthy subjects (14 pmol/l). Copeptin levels in the remaining group of patients was not significantly different to the control group.
Our data support the idea that measurement of serum copeptin levels may help to rapidly categorize SIADH as the cause of hyponatraemia in cancer patients as compared to other causes of hyponatraemia. These determinations may guide the decision for treatment with blockers of the AVP receptor.

P248
Thyroid function in patients with pituitary disease: difficulties of individualisation
Ahmed El-Laboudi1,2, Julie Lynch1,2, Paul Baxter1,2, Julian Barth1,2 & Robert Murray1,2
1Teaching Hospitals NHS Trust, Leeds, West yorkshire, UK; 2University of Leeds, Leeds, West Yorkshire, UK.

Introduction
Identification and treatment of central hypothyroidism in hypopituitarism is crucial particularly in patients with significant lethargy under consideration for GH replacement. Early Identification of TSH deficiency can be challenging particularly when both the TSH and fT4 lie within the normal range.
Methods
26 362 TFTs derived from ambulatory community dwelling individuals represented the control group. TFTs from 227 patients with a putative or established insult to the hypothalamo-pituitary axis were studied; 145 from patients with central hypothyroidism on levothyroxine replacement and 82 samples from patients with hypopituitarism without a diagnosis of central hypothyroidism.
Results
Median fT4 (5th and 95th percentiles) value for the control group was 14.3 (10.9–19.5) pmol/l. Hypopituitary patients without a formal diagnosis of secondary hypothyroidism showed median fT4 13.1 (9.5–16.5) pmol/l, significantly lower than in the control group (P<0.001). Of these individuals 11% showed fT4 levels below the 5th percentile of the control group. Graphically, a left shift of the fT4 distribution curve was observed in this subgroup. The degree of hypopituitarism, defined by the number of pituitary hormone deficits, had no effect on the median fT4 level within this subgroup. In hypopituitary patients with a diagnosis of secondary hypothyroidism, and established on levothyroxine replacement, the median fT4 was 16.3 (11.1–23.7) pmol/l representing significantly higher values than observed in the control group (P<0.001).
Graphically, the distribution curve for these individuals showed a wider base and lower peak frequency.
Conclusion
These data suggest that a subtle decrease in fT4 is present in a relatively large proportion of patients who receive a putative insult to the hypothalamo-pituitary axis. In the majority of these individuals fT4 values remain well within the normative range. Furthermore, examination of fT4 values of patients on levothyroxine shows difficulties with placement of the fT4 and potential over-treatment in a number of individuals.

P249
Prospective assessment of pituitary function in patients with macroprolactinoma treated with cabergoline
Niki Karavitaki, Ruxandra Dobrescu & John Wass
Department of Endocrinology, Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK.

Background
Patients with macroprolactinoma often present with pituitary hormone deficits associated with hyperprolactinaemia or mass effect. Restoration of normoprolactinaemia and tumour shrinkage by dopamine agonist is expected to reverse, at least partially, the pituitary dysfunction. Studies assessing prospectively the pituitary function in subjects with macroprolactinoma treated with cabergoline are lacking.

Aim
To check the time course of recovery of the anterior pituitary reserve in patients with macroprolactinoma responding to cabergoline.

Patients/Methods
All patients presenting to our Department with macroprolactinoma between 10/2005-10/2007 were studied prospectively. The subjects underwent assessment...
of their pituitary function at diagnosis and, if deficient, at yearly intervals. Serum prolactin was checked at regular intervals during the titration of the dose of cabergoline and 6 monthly after the achievement of normoprolactinaemia. Pituitary imaging was performed 3 months after starting treatment and at yearly intervals thereafter.

Results

Thirteen patients were identified, of which 12 were lost to follow-up (final group 11 males and 1 female, median age at diagnosis 40.5 years (range 17–81), mean serum prolactin at diagnosis 27.547 mM (range 17.168–305.847)). Amongst those with pituitary hormone deficits, mean follow-up was 2.9 yrs (range 2–3). Normal prolactin was achieved in 11 and tumour shrinkage in 11 subjects. Hormone deficits at diagnosis and at last evaluation were GH (severe): 10/12 (83%) and 10/12 (83%), FSH/LH: 10/12 (100%) and 6/10 (60%) (female on oral contraceptive pill and male on Leuprolein Acetate excluded), ACTH: 2/12 (17%) and 2/12 (17%), TSH: 4/11 (36%) (patient with primary hypothyroidism excluded) and 4/11 (36%).

Conclusions

In this first prospective study of pituitary function in treated macroadenoma subjects, apart from gonadotroph axis recovery (probably related to the treatment of hyperprolactinaemia), we found no improvement of pituitary hormone deficits, even after tumour shrinkage. Therefore, relevant replacement therapy should be commenced at the outset of dopamine agonist treatment.

P250

Delivery of anti-POMC siRNA using a novel polymer

Helen Prescott1, Alia Munir1, Irene Canton2, Giuseppe Battaglia1, & John Newell-Price1

1University of Sheffield, Sheffield, UK; 2Tissue Engineering, University of Sheffield, Sheffield, UK; 3Department of Biomedical Science, University of Sheffield, Sheffield, UK.

Background

Previously, we have demonstrated highly potent knockdown of POMC mRNA and significant reduction in secreted ACTH using siRNA, both in vitro and in vivo. Highly efficient delivery of siRNA to a target tissue is of critical importance for any therapeutic approach. Here we assess the suitability of a pH-sensitive polymer, PMPC-PDPA polymersomes encapsulating siRNA, as a novel delivery system as these have potential for tissue-directed delivery by co-complexing with appropriate antibodies, with intracellular release dependent on pH change after uptake.

Methods

The lowest effective dose of siRNA targeting POMC was assessed in vitro in AtT20 cells using a commercial lipid delivery agent. PMPC-PDPA polymersomes were devised and optimized used to encapsulate the siRNA and the morphological characteristics determined. Immunofluorescence was used to investigate the presence of corticotrophin releasing hormone receptor 1 (CRHR1) on the surface of AtT20 cells.

Results

Suppression of POMC mRNA was demonstrated in a dose dependent manner. A 1 nM concentration of siRNA achieved a 50% reduction in ACTH compared to a negative siRNA control (P < 0.001). Electron microscopy revealed PMPC-PDPA polymersomes encapsulating siRNA had a diameter of 100 nm, whilst having a neutral surface charge – consistent with these particles having characteristics suitable for in vivo delivery. Real time quantitative PCR demonstrated the expression of CRHR1 mRNA in AtT20 cells and immunofluorescence demonstrated the presence of CRHR1 on the surface of AtT20 cells.

Conclusion

Polymersome-encapsulated siRNA targeting POMC have potential as a therapeutic for Cushing’s disease, with corticotroph targeting possible by antibody-particle complexes.

P252

An uncommon cause of panhypopituitarism

Uppendra Srinivas-Shankar1, Samudra Bujawansa1, Niamh Leonard2, Peter Clark3, Isabel Syndikus3, Leigh Forsyth1 & Sian Hickey1

1St Helens and Knowsley Teaching Hospital NHS Trust, St Helens, UK; 2Royal Liverpool University Hospital NHS Trust, Liverpool, UK; 3Clatterbridge Centre for Oncology NHS Trust, Clatterbridge, UK.

Introduction

Langerhan’s Cell Histiocytosis (LCH) is a rare disease, more common in children than in adults, resulting from aberrant proliferation of Langerhan’s cells, belonging to the monocyte–macrophage system.

Case history

We present the case report of a 40-year-old man with a 16-year history of polypria, polydipsia and tiredness. For 10 years he had perianal, groin, abdomen and scalp scarring, hyperpigmentation along with follicles and pustules. Skin biopsy confirmed LCH. Twenty-four hour urine volume excursion was over 4 l and water deprivation test confirmed diabetes insipidus (DI). He also had secondary adrenocortical insufficiency (short synacthen test: baseline and 30-min cortisol, 92 and 263 nmol/l respectively) and hypogonadotrophic hypogonadism (FSH, LH were 1.0 and 0.4 IU/l respectively; Total testosterone 3.9 mg/ml). IGF1 was low (8 nmol/l; N= 12−47). Thyroid function tests and serum prolactin level were normal. Magnetic resonance imaging (MRI) scan of the pituitary confirmed loss of high signal of the posterior pituitary on T1 enhancement and marked enhancement of the hypothalamus. Although lung function tests revealed restrictive pattern, High Resolution CT scan of the chest excluded pulmonary involvement. DEXA scan revealed osteopenia (lumbar spine and left femoral neck, T score −2.1 and −1.9 respectively). He was treated with desmopressin, oral hydrocortisone and testosterone, with significant improvement of symptoms. Radiotherapy was considered but was deferred as subsequent MRI scan showed improvement of the hypothalamic lesion.

Conclusion

Hypothalmo-pituitary axis involvement occurs in up to 50% of patients with LCH; unexplained skin and mucosal lesions of the scalp and intertriginous areas with polyuria should prompt clinicians to consider this condition. Although DI is the earliest and commonest pituitary manifestation (25%) of LCH, up to 20% may have anterior pituitary involvement; long-term follow up is needed to detect endocrine deficiencies.

P253

Associations of overall GH and IGF1 exposure with ischaemic heart disease and cardiomyopathy in patients with treated acromegaly

Holly Clarke, Channa Jayasena, Alexander Comninos, Mandy Donaldson, Karim Meeran & Waljit Dhillo

Imperial College London, London, UK.

Background

Patients with acromegaly require lifetime monitoring due to the excess mortality and morbidity associated with untreated disease, and the propensity for disease...
relapse following treatment. There is controversy whether GH or insulin-like growth factor 1 (IGF1), better predicts the onset of cardiovascular complications such as cardiomyopathy and ischaemic heart disease (IHD) in acromegalic patients.

Aim
To examine associations of overall GH and IGF1 exposure with IHD and cardiomyopathy, in patients with treated acromegaly.

Methods

Records of 116 patients with treated acromegaly attending a single Endocrine centre were examined retrospectively. GH and IGF1 burdens were calculated by multiplying the overall mean basal GH and mean IGF1 index during patient follow-up, by the number of years since diagnosis of acromegaly. IGF1 index was defined as serum IGF1 divided by the upper limit of reference range. Mean GH and IGF1 burdens were compared between patients with and without cardiomyopathy and IHD.

Results

IHD was present in 11.2% of treated acromegalic patients. GH burden was significantly higher in patients with IHD when compared with patients without IHD (mean GH burden in years: 20.6±3.6 vs. 15.2±2.9; IHD; *P* = 0.009). Mean IGF1 burden was not significantly different between patients with and without IHD. Evidence of cardiomyopathy was recorded in 20% of treated acromegalic patients. Mean IGF1 burden was significantly higher in patients with cardiomyopathy when compared with patients without cardiomyopathy (mean IGF1 burden in years: 23.5±4.4, cardiomyopathy; 16.2±1.6, no cardiomyopathy. *P* = 0.011). Mean GH burden was not significantly different between patients with and without cardiomyopathy.

Conclusion

These results suggest that both GH burden and IGF1 burden are useful markers of cardiovascular morbidity in treated acromegalic patients. This study highlights the importance of monitoring both serum GH and IGF1 in treated acromegalic patients.

P255

**Morphological analysis of lactotrophs in pregnant and lactating mice**

Joe Lockey, John Morris & Helen Christian

University of Oxford, Oxford, UK.

Recent studies have demonstrated that rather than being a collection of heterogeneously dispersed cells, the pituitary gland is wired by multiple and specific endocrine cell networks to synchronise hormone release. Prolactin (PRL) is primarily important for lactation. In response to changing physiological demands during pregnancy and lactation the pituitary has the ability to expand and contract its cell number. We have investigated changes in lactotroph morphology and cell-to-cell contacts in virgin, pregnant and lactating mice. Anterior pituitary sections from virgin, 1 week pregnant (1P), 3 week pregnant (3P) and lactating mice (n=4 per group) were immunogold labelled for PRL and examined by quantitative electron microscopy to determine lactotroph size, secretory granule characteristics and rough endoplasmic reticulum (RER) density. The identity of cell types contacting lactotrophs were also quantified. Lactotroph number was increased in lactation but cytoplasmic area was not significantly different between groups. Secretory granule density was significantly (*P*<0.05) increased in 1P mice but decreased in 3P (*P*<0.01) and the density of RER was significantly greater in lactating and 1P and 3P groups compared to virgin consistent with greater secretory activity. In virgin mice, lactotrophs made junctional contacts with all the major classes of pituitary endocrine cell types but more with GH, PRL and folliculo-stellate cells, and only a few ACTH, LH and TSH cells which probably reflects the relative proportion of the cell types in the pituitary. In lactation there was a significant increase (*P*<0.01) in the number of contacts made with other lactotrophs and a significant decrease (*P*<0.01) in contacts made with folliculo-stellate cells. Folliculo-stellate cells release a number of factors inhibitory to PRL release, such as annexin 1, and these data suggest that increased lactotroph connectivity coupled with a retraction of the folliculo-stellate cell network contributes to increased PRL secretion in lactation.

---

**P254**

**Overall GH exposure is raised in acromegalic patients with type 2 diabetes and impaired glucose tolerance, when compared with euglycaemic acromegalic patients**

Holly Clarke, Channa Jayasena, Alexander Comninos, Mandy Donaldson, Karim Meenan & Waljit Dhillo

Imperial College London, London, UK.

**Background**

A cardinal feature of acromegaly is insulin resistance. Patients with acromegaly are therefore predisposed to developing impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM). It is therefore imperative to develop better biomarkers predicting the onset of IGT and T2DM in treated acromegalic patients. There is controversy whether GH or insulin-like growth factor 1 (IGF1) better predict the onset of IGT or T2DM in treated acromegalic patients. However the associations of overall GH and IGF1 exposure in patients with treated acromegaly have not been investigated previously.

**Aim**

To compare overall GH and IGF1 exposure in treated acromegalic patients classified according to the presence or absence of IGT and T2DM.

**Methods**

Records of 116 patients with treated acromegaly attending a single endocrine centre were examined retrospectively. T2DM and IGF were diagnosed using a 75g oral glucose tolerance test. GH and IGF1 burdens were calculated by multiplying the overall mean basal GH and mean IGF1 index during patient follow-up, by the number of years since diagnosis of acromegaly. IGF1 index was defined as serum IGF1 divided by the upper limit of reference range. Mean GH and IGF1 burdens were compared between euglycaemic patients, and patients with IGT or T2DM.

**Results**

IGT and T2DM were present in 28 and 27% of treated acromegalic patients, respectively. The mean GH burden was significantly lower in euglycaemic patients when compared with patients with IGT or T2DM (mean GH burden in mcg/L: 46.1±5.2, euglycaemic; 80.7±20.1, IGT, *P* = 0.01 versus euglycaemic; 65.0±13.0, T2DM, *P* < 0.05 versus euglycaemic). Mean IGF1 burdens were not significantly different between patient groups.

**Conclusion**

These results suggest a strong association between overall GH exposure and abnormal glucose tolerance in patients with treated acromegaly. GH burden may therefore provide a useful prognostic marker in predicting the onset of IGT or T2DM in treated acromegalic patients.

---

**P256**

**Hypertrophic pachymeningitis and pituitary pathology: lymphocytic hypophysitis or cabergoline related fibrosis?**

Rajeev Raghavan1, Elizabeth Mallam2, Neil Scolling1,2 & Karin Bradley1,3

1Department of Endocrinology, University Hospitals Bristol NHS Foundation Trust, Bristol Royal Infirmary, Bristol, UK; 2Department of Neurology, North Bristol NHS Trust, Frenchay Hospital, Bristol, UK; 3University of Bristol, Bristol, UK.

**Case history**

A 51-year-old lady with a background of classical migraine and amenorrhea conceived successfully via IVF, without prior endocrine assessment. Symptoms during the third trimester of pregnancy led to the diagnosis of a pituitary mass lesion (see Table). Possible differential diagnoses, on subsequent endocrine review, included macroprolactinoma, microprolactinoma with expansion during pregnancy, or lymphocytic hypophysitis. After 6 years of dopamine agonist therapy, the patient was then admitted with progressive generalised headaches and vomiting (similar to her symptoms during pregnancy and different from her usual migraine). Neurological assessment, including a comprehensive autoimmune workup and gallium scan, were all unremarkable. CSF analysis was normal except for a mildly elevated protein.

**Table**

<table>
<thead>
<tr>
<th>Time-line</th>
<th>History</th>
<th>Prolactin (&lt;700 mIU/L)</th>
<th>MR imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>Secondary amenorrhoea</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2002</td>
<td>Infertility clinic/IVF therapy</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2002</td>
<td>Pregnancy 36 weeks--significant headaches, small left temporal focal defect</td>
<td>–</td>
<td>Macroadenoma</td>
</tr>
<tr>
<td>2002</td>
<td>Rapid resolution of field defect pospartum without intervention</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2003</td>
<td>Persistent amenorrhoea 6 months pospartum, Cabergoline started</td>
<td>3818</td>
<td>Macroadenoa unchanged</td>
</tr>
<tr>
<td>2004</td>
<td>Cabergoline 500 μg/week</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2008</td>
<td>Cabergoline reduced to 250 μg/week</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2009</td>
<td>Disabling headaches</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>2010</td>
<td>Pachymeningitis diagnosed, Cabergoline weaned and stopped 07/2009</td>
<td>41</td>
<td>Pituitary appearance unchanged</td>
</tr>
</tbody>
</table>

---

Endocrine Abstracts (2011) Vol 25
A repeat MRI demonstrated new diffuse meningeal thickening. Hypertrophic pachymeningitis (patchy widespread inflammatory thickening of the dura mater) was diagnosed.

Conclusion
We report the rare association of pituitary pathology, treated with an ergot-derived dopamine agonist, and hypertrophic pachymeningitis. Withdrawal of the cabergoline (implicated in cardiac valvular fibrosis) coincided with rapid resolution of the clinical and radiological features associated with pachyme ningitis. Whilst this could be purely coincidental, a causal link cannot be definitively excluded.

P257
Thyroid function in patients with pituitary disease: difficulties of individualisation
Ahmed El-Laboudi1, Julie Lynch1, Paul Baxter2, Julian Barth3 & Robert Murray1
1Department of Endocrinology, Leeds Teaching Hospitals NHS Trust, Leeds, UK; 2Department of Statistics, University of Leeds, Leeds, UK; 3Department of Clinical Biochemistry, Leeds Teaching Hospitals NHS Trust, Leeds, UK.

Introduction
Identification and treatment of central hypothyroidism in hypopituitarism is crucial particularly in patients with significant leythargy under consideration for GH replacement. Early Identification of TSH deficiency can be challenging particularly when both the TSH and fT4 lie within the normal range.

Methods
26 362 TFTs derived from ambulatory community dwelling individuals represented the control group. TFTs from 227 patients with a putative or established insult to the hypothalamo-pituitary axis were studied; 145 from patients with central hypothyroidism on levothyroxine replacement and 82 samples from patients with hypopituitary thyroiditis without a diagnosis of central hypothyroidism.

Results
Median fT4 (5th and 95th percentiles) value for the control group was 14.3 (10.9–19.5) pmol/l. Hypopituitary patients without a formal diagnosis of secondary hypothyroidism showed median fT4 13.1 (9.5–16.5) pmol/l, significantly lower than in the control group (P<0.001). Of these individuals 11% showed fT4 levels below the 5th percentile of the control group. Graphically, a left shift of the fT4 distribution curve was observed in this subgroup. The degree of hypothyroidism, defined by the number of pituitary hormone defects, had no effect on the median fT4 level within this subgroup. In hypopituitary patients with a diagnosis of secondary hypothyroidism, and established on levothyroxine replacement, the median fT4 was 16.3 (11.1–23.7) pmol/l representing significantly higher values than observed in the control group (P<0.001). Graphically, the distribution curve for these individuals showed a wider base and lower peak frequency.

Conclusion
These data suggest that a subtle decrease in fT4 is present in a relatively large proportion of patients who receive a putative insult to the hypothalamo-pituitary axis. In the majority of these individuals fT4 values remain well within the normal range. Furthermore, examination of fT4 values of patients on levothyroxine shows difficulties with placement of the fT4 and potential overtreatment in a number of individuals.

P258
An unusual presentation of thyrotroph adenoma
Rajeev Raghvan1, Puneet Plaha1, Richard Nelson2, Stafford Lightman1,3 & Andrew Levy2,3
1Department of Endocrinology, Bristol Royal Infirmery, University Hospitals Bristol NHS Foundation Trust, Bristol, UK; 2Department of Neurosurgery, Frenchay Hospital, North Bristol NHS Trust, Bristol, UK; 3Henry Wellcome Laboratory for Integrative Neuroscience and Endocrinology, University of Bristol, Bristol, UK.

Background and case
A 38-year-old man presented acutely with left-sided retro-orbital pain, a heavy/numb sensation in his left arm and blurred vision and diplopia on left lateral gaze. Baseline biochemistry, liver function and haematology were normal. His TSH was 3.9 (0.3–4 mU/l) and CRP 92 mg/l (<10). Plain CT head was unremarkable as was the MRI as reported, and atypical migraine was suspected.

Vertical diplopia on left lateral gaze and left upper arm paraesthesia persisted, however, and 4 months later, following repeat normal baseline bloods and normal CSF examination, a repeat MRI head demonstrated a 12×12 mm pituitary adenoma that was unchanged but had been overlooked on initial MRI.

Endocrine review
He was phenotypically normal with full visual fields. The only additional history was of anger and mood disturbance. Baseline pituitary function revealed a TSH of 4.5 mU/l with a FT4 of 28.5 pmol/l (10–24) and FT3 of 7.6 pmol/l (2.8–7.1). Random PRL was 198 mU/l (<700), testosterone 20.6 nmol/l (10–35) and GH 0.1 µg/l.

Management
Almost one year after his initial presentation and following 4 weeks of pre-operative preparation with cabimazole (15 mg/day), a transsphenoidal microadenomectomy for thyrotrophinoma was carried out. Post-operative FT4 and FT3 were 20.8 and 5.8 pmol/l respectively, with a TSH of 2.4 mU/l. Immunohistochemistry showed predominantly GH immunostaining, with weak, scattered TSH positivity.

Discussion
The involvement of the left trochlear nerve is difficult to explain unless some degree of vascular shunting is added. The thyrotrophinoma did not encroach into the cavernous sinuses, however, and such a mechanism could certainly not explain the left arm symptoms. This case is a reminder that pituitary pathology which is generally straightforward to exclude, should be considered in the differential diagnoses of atypical headache, and that pituitary adenomas may cause very unusual neurological symptoms.

P259
Wide range of eye abnormalities in patients with hypopituitarism; implications for diagnosis and treatment
Kyriali S Alatzoglou1, Daniel Kelberman1, Emanuella Spadoni2, Carles Gaston-Massuet1, Kathryn Woods1, Mohamad Maghnihne1, Maria Bittner-Glindzicz1 & Mehul T Dattani1
1Developmental Endocrinology Research Group, UCL Institute of Child Health, London, UK; 2Clinical and Molecular Genetics Unit, UCL Institute of Child Health, London, UK; 3Department of Paediatrics, University of Pavia, Pavia, Italy.

Background and aim
The development of the pituitary gland is closely linked to this of the eyes and forebrain, as they all originate from the same embryonic origin, the anterior neural ridge. The constellation of symptoms leading to septo-optic dysplasia (SOD) is well established; other ophthalmic signs may be under-reported. The aim of this study was to define if patients with hypopituitarism present with eye abnormalities, which are distinct from SOD, and if this association could help the genetic diagnosis of the condition.

Methods
We studied case records of patients referred over the last 10 years from national and international centres.

Results
We identified 96 patients with hypopituitarism (male:female 1:2:1), who had eye abnormalities that were distinct from SOD. Hypopituitarism was familial in 14.5% (n=14) and in 5.2%, patients were of consanguineous pedigrees. The spectrum of eye abnormalities included: colobomas not associated with CHARGE syndrome (n=11, 1.5%), pigmented disorders of the retina (n=4, 4.2%), retinal dysplasia (n=13, 13.5%), congenital cataracts, (n=3, 3.5%), amaurosis (n=3, 3.5%), anophthalmia or microphthalmia (n=32, 33.3%). Other eye abnormalities (n=30, 31.2%), ranged from glaucoma to blepharophimosis, staphylomas and atypical appearance of the optic discs. In four cases with retinal pigment defects or retinal dysplasia there was a single pituitary hormone deficiency, isolated GHD (n=2). In all other cases, retinal abnormalities were associated with multiple pituitary hormone deficiencies. SOX2 mutations were identified in 40% (13/32) of patients with anophthalmia or microphthalmia and they all had gonadotrophin deficiency; we identified a novel OTX2 mutation (p.E79X) in one a patient. We found no mutations in HESX1, GHIH, SIX3, PAX6 or SOX3 in this cohort.

Conclusions
Patients with hypopituitarism may present with variable eye abnormalities. Monitoring for pituitary hormone deficiencies is recommended in children with congenital eye abnormalities. The study of the association of eye and pituitary abnormalities may help to identify novel genes implicated in pituitary development.
Abstract withdrawn.

P261

Lymphocytic hypophysitis–extrapancreatic manifestation of autoimmune pancreatitis

Damodharan Suresh & Gerard Conway
UCLH, London, UK.

Background

Auto-immune pancreatitis (AIP) is a rare chronic inflammatory disease, characterised by raised serum levels of IgG4, which may mimic pancreaticobiliary malignancy, and is noted to have an IgG4-positive plasma cell infiltrate on pancreatic histology. Extrapancreatic manifestations in liver, kidneys, and retroperitoneum, are increasingly recognised.

We present a case of extrapancreatic manifestation of AIP in the pituitary gland causing lymphocytic hypophysitis leading to panhypopituitarism. Case history

A 67-year-old man presented with obstructive jaundice. A diagnosis of AIP/IgG4-associated choanalitis was made based on imaging, serology and biopsy. Oral steroids and azathioprine led to resolution of jaundice and normalisation of liver function tests. Steroids were weaned and stopped over 6 months with maintenance azathioprine. He presented again with weakness and malaise. Examination revealed heart rate 80 bpm, blood pressure 80/40 mmHg, but no other signs of note.

Investigations

Table 1 Results and clinical impression of adrenal insufficiency.

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Results</th>
<th>Normal range</th>
<th>Impression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Random cortisol</td>
<td>&lt;20 mol/l</td>
<td>&lt;140 nmol/l</td>
<td>Adrenal insufficiency (assumed secondary to prolonged steroid use)</td>
</tr>
</tbody>
</table>

Table 2 Further endocrine profile.

<table>
<thead>
<tr>
<th>Investigations</th>
<th>Results</th>
<th>Normal range</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSH</td>
<td>0.7 mIU/L</td>
<td>0.27–4.2 mIU/L</td>
<td>Panhypopituitarism</td>
</tr>
<tr>
<td>T4</td>
<td>7.2 pmol/l</td>
<td>12–22 pmol/l</td>
<td></td>
</tr>
<tr>
<td>LH</td>
<td>&lt;0.1 IU/L</td>
<td>1.7–8.6 IU/L</td>
<td></td>
</tr>
<tr>
<td>FSH</td>
<td>0.8 IU/L</td>
<td>1.5–12.4 IU/L</td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td>0.1 nmol/l</td>
<td>9.9–27.8 nmol/l</td>
<td></td>
</tr>
<tr>
<td>Serum osmolality</td>
<td>300 mOsm/kg</td>
<td>285–295 mOsm/kg</td>
<td></td>
</tr>
<tr>
<td>Urine osmolality</td>
<td>263 mOsm/kg</td>
<td>300–900 mOsm/kg</td>
<td></td>
</tr>
</tbody>
</table>

Gadolinium-enhanced MRI showed significant enlargement and infiltration of the pituitary stalk, consistent with inflammation. Hormone replacement led to resolution of symptoms. Prednisolone 30 mg was re commenced with complete resolution of the pituitary infiltrate on repeat gadolinium MRI 6 weeks later. He remained clinically well.

Autoimmune pancreatitis is now recognised as a disease which can occur globally. Extra-pancreatic disease is increasingly recognised as a common feature, and there have been two other case reports of lymphocytic hypophysitis in AIP. Early recognition of this is important in rational management of these patients.

Discussion

Difficulties encountering in the investigations of his Cushing’s syndrome. Would we consider alternative medical therapy? And if after exhaustive investigation, we failed to find a source of the ACTH, then bilateral adrenalectomy could be considered!

P262

Elusive ectopic ACTH source

Sami Kenz, S McGlynn, Daniel Kannappan & Tara Kearney
Salford Royal Foundation NHS Trust, Manchester, UK.

Introduction

The ectopic ACTH syndrome accounts for 12% of patients with Cushing’s syndrome. Its diagnosis and treatment remains a challenge. This especially true in patients with ectopic ACTH production.

Case

We report the case of a 41-year-old man who presented with progressive muscle weakness in his arms and legs. He was found to be borderline hypertensive BP 145/92, cushingoid plethoric rounded face and mild proximal muscular weakness. His initial investigations revealed high serum cortisol of 931 and low K 3.4. Further investigations revealed very high urinary free cortisol excretion 2454 and high urinary cortisol 944. Cortisol failed to suppress on both low dose and high dose dexamethasone tests 835 and 621 respectively. MRI pituitary with contrast (dynamic) shows a small area of non-enhancement at the base of pituitary stalk. The pituitary fossa itself was normal. IPSS failed to show a definite gradient and was equivocal (19 on the left, 22 on the right, 17 peripherally), and after CRH administration there was no discernable rise. CT chest and abdomen revealed no ectopic source of ACTH. His Octreotide scan was also normal with no evidence of abnormal uptake.

He became more symptomatic especially with regards to his proximal myopathy. He was then started on metyrapone. However, he was not able to tolerate metyrapone and it was stopped. Pituitary surgery was not considered as his MRI findings were not entirely convincing.

Reproduction

P263

Endocrine disruptors and their association with male reproductive disorders and testicular dysgenesis syndrome: establishing a xenografting model of human fetal testis development

Rod Mitchell1,3, Philippa Saunders1, Andrew Childs2, Claire Cassidy-Kojima1, Richard Anderson2, Hamish Wallace2, Chris Kelman2, & Richard Sharpe1
1MRC Human Reproductive Sciences Unit, Edinburgh, UK; 2Edinburgh Royal Hospital for Sick Children, Edinburgh, UK; 3Edinburgh University, Edinburgh, UK.

Testicular dysgenesis syndrome (TDS) is a group of associated conditions (testicular germ cell tumours (TGCT), cryptorchidism, hypospadias and low sperm counts) that are thought to have a common origin in fetal life. Exposure of fetal rats to environmental chemicals such as the endocrine disrupting chemical di(2-ethylhexyl) phthalate (DBP) results in a TDS-like syndrome. However, exposed rats do not develop TGCT and the rodent is a poor model in this context. The effects of phthalates on human fetal testis development and function are difficult to study in the short-term in vivo. Development of a model system in which to investigate normal human fetal testis development and the mechanisms underpinning the fetal origin of TGCT/TGCT cells would represent a major advance.

We have, therefore, xenografted human fetal testis tissue from first and second trimester fetuses into nude mice hosts for 6 weeks and demonstrated normal development of tissue (including normal seminiferous cord formation) in comparison to age-matched controls. Xenografts continue to grow, and the component cells proliferate and functionally differentiate normally. Xenografts are able to produce basal levels of testosterone that can be increased by injection of host mice with hCG. Exposure of xenografts to DBP for 24–96 h by daily oral gavage results in germ cell aggregation and formation of multinucleated gonocytes, whereas GC aggregation was not demonstrated in vehicle treated controls and MNG were found infrequently.

These results have validated an exciting and novel way in which to investigate the mechanisms of human fetal testis development in health and disease. Importantly, this model may also be applied to study the effects of other environmental factors on human testis development. Results of such studies will help to determine the risk posed to human health by exposure to these chemicals during a critical time in gonadal development.

P264

Distinct expression pattern of Dicer1 correlates with ovarian-derived steroid hormone receptors in human Fallopian tubes during the ovulatory process and in the mid-secretory phase

Ruijin Shao & Hakan Billig
Institute of Neuroscience and Physiology, The Sahlgrenska Academy at the University of Gothenburg, Göteborg, Sweden.

Context

Tissue-specific dicer1 knockout female mice display severe and irreversible damage to the Fallopian tubes that eventually leads to disruption of tubal transport
of the gametes and the early embryo. The impact of Dicer1 on the function of human Fallopian tubes remains to be clarified.

**Objective**

To determine how the expression of tubal Dicer1 is regulated in women during the ovulatory process and in the mid-secretory phase. Furthermore, the association between tubal Dicer1 expression and alterations in estrogen receptor (ER) subtype and progesterone receptor (PR) isoform expression was investigated.

**Research design and methods**

The three phases of the ovulatory process were defined as followed: (1) the early ovulatory phase \( (n=4) \); (2) the late ovulatory phase \( (n=4) \); and (3) the post-ovulatory phase \( (n=5) \). The mid-secretory phase \( (n=4) \) was determined by the last menstrual period and endometrial histology. Serum was obtained immediately before surgery to confirm the ovulatory and mid-secretary phase categories. Localization and regulation of Dicer1, ER subtypes, and PR isoforms were determined by immunofluorescence, confocal microscopy and quantitative RT-PCR. Correlations were evaluated by bivariate Pearson’s correlation coefficients.

**Results**

Dicer1 protein expression was most abundant in epithelial cells of the Fallopian tubes. The expression levels of Dicer1 mRNA and protein were significantly higher in the late ovulatory phase than in other phases. Moreover, no significant relationship between stages in ER subtype and PR isoform mRNA levels was found during the ovulatory process; however, expression levels of ERβ1 and ERβ2 mRNA and protein were significantly lower in all phases of the ovulatory process compared to the mid-secretory phase. The opposite pattern was seen in the expression levels of PRA/B and PRB mRNA and protein. Dicer1 mRNA expression was positively correlated with expression of ERα mRNA in the late ovulatory phase and negatively correlated with expression of ERβ2 mRNA in the mid-secretory phase, as well as PRB mRNA in the early ovulatory phase.

**Conclusion**

This is the first study to identify the cell-specific upregulation of Dicer1 expression in human Fallopian tubes during the ovulatory process. While the regulation of steroid hormone receptors is well established, stage-dependent expression of Dicer1 and its correlation to ERα, ERβ2 and PRB mRNA suggest that tubal Dicer1 may participate in the regulation of tubal steroid hormone receptor expression in a cycle-dependent manner and may be an important contributor to the tubal transport process in humans.

Key words: Dicer, microRNA, steroid hormone receptor, Fallopian tube, human ovary.

---

**P265**

**The effect of atorvastatin on adrenal and ovarian hyperandrogenemia in patients with polycystic ovary syndrome.**

Thozhukat Sathyapalan1, Karen A Smith2, Anne-Marie Coady3, Eric S Kilpatrick2 & Stephen L Atkin1

1Department of Academic Endocrinology Diabetes and Metabolism, Hull York Medical School, Hull, UK; 2Department of Clinical Biochemistry, Hull Royal Infirmary, Hull, UK; 3Department of Obstetric Ultrasound, Hull and East Yorkshire Women’s and Children’s Hospital, Hull, UK.

**Context**

Hyperandrogenemia in polycystic ovary syndrome (PCOS) represents a composite of raised serum concentrations of testosterone, androstenedione, dehydroepiandrosterone (DHEA), and DHEA sulfate (DHEAS). In patients with PCOS, testosterone and androstenedione are primarily derived from the ovaries and DHEAS is a metabolite predominantly from the adrenals. It has been shown that atorvastatin reduces testosterone levels in patients with PCOS.

**Objective**

This randomized double blind placebo controlled study was conducted to study the effects of atorvastatin on serum androstenedione and DHEAS concentrations in patients with PCOS.

**Intervention**

Forty medication naïve patients with PCOS were randomized to either atorvastatin 20 mg daily or placebo for 3 months. Subsequently, a 3 month extension study for all patients was undertaken with metformin 1500 mg daily. The main outcome measures were the changes in androstenedione and DHEAS concentrations.

**Results**

The mean (±SD) baseline androstenedione (5.6 (9.9) vs 5.5 (1.3) mmol/l; \( P=0.58 \)) and DHEAS (7.1 (1.0) vs 7.2 (1.2) mmol/l; \( P=0.72 \)) levels were comparable between two groups. There was a significant reduction of androstenedione (5.6 (0.9) vs 4.7 (0.7) mmol/l; \( P=0.003 \)) and DHEAS (7.1 (1.0) vs 6.0 (0.9) mmol/l; \( P=0.02 \)) with atorvastatin compared to placebo. Three months treatment with metformin maintained the reduction of androstenedione and DHEAS levels with atorvastatin compared to baseline. There were no changes in either DHEAS or androstenedione concentrations in the initial placebo group after 12 weeks of metformin.

**Conclusions**

Twelve weeks of atorvastatin significantly reduced both DHEAS and androstenedione contributing to the total reduction of androgen levels and indicating that the reduction of the hyperandrogenaemia is due to the action of atorvastatin at both the ovary and the adrenal gland in PCOS.

---

**Table 1.**

<table>
<thead>
<tr>
<th>Site</th>
<th>D3 activity (fmol/min per mg protein) median (interquartiles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near</td>
<td>7.6 (4.3, 12.5)</td>
</tr>
<tr>
<td>Middle</td>
<td>4.9 (3.3, 8.3)</td>
</tr>
<tr>
<td>Outer</td>
<td>4.8 (3.2, 7.5)</td>
</tr>
</tbody>
</table>

---

**P266**

**Placental iodothyronine deiodinase activity across the placental bed in normotensive (NT) and pre-eclamptic (PE) pregnancies.**

Lesia Kural1, Eileen Mistry2, Ellen Kaptein1, Theo Visser1 & Fiona Broughton Pipkin1

1Department of Obstetrics and Gynaecology, University of Nottingham, Nottingham, UK; 2Division of Women’s Health, King’s College London, London, UK; 3Department of Internal Medicine III, Erasmus University Medical School, Rotterdam, The Netherlands.

**Background**

PE affects ~2% of pregnancies and is associated with generalised endothelial cell dysfunction. Antioxidant selenoproteins may limit such effects. Serum selenium is lower in mothers and babies affected by PE and associated with lower activity of the selenoprotein glutathione peroxidases. Other selenoproteins, the iodothyronine deiodinases (IDs; D2 and D3) regulate fetal thyroid hormone synthesis. The IDs utilise selenium preferentially; we have reported similar triiodothyronine (T3) and thyroxine (T4) in both maternal and umbilical serum from NT and PE pregnancies. We hypothesised that ID enzyme activities would be conserved in PE placenta.

**Design**

Hospital based cross-sectional study with full ethics approval. Placental biopsies were obtained at delivery from 3 sites; 1 cm from cord insertion (near), midway between insertion and edge (middle) and 1 cm from the edge (outer) from 27 NT and 21 PE European women. Homogenate D2 and D3 activities were determined by HPLC analysis following formation of the radioactive T4 and T3 respectively. Protein localisation was confirmed by immunohistochemistry.

**Results**

D3 enzyme activity was identified in all placenta; immunoreactivity was confirmed in the syncytiotrophoblasts. Friedman – Repeated Measures demonstrated a significant \( P=0.034 \) gradient in placental D3 activity (Table 1) in NT but not PE. Activity in individual sampling sites did not differ between NT and PE. Neither D2 protein expression nor activity were detected.
Conclusions
These data show no effect of PE at delivery on placental D3 activity which corroborates our earlier data showing comparable mRNA expression and similar (Tα) and (Tβ) in NT and PE samples. The difference in activity across the placental bed only in NT pregnancy suggests a possible blunting in enzyme regulation in PE which may relate to the lower tissue oxygenation at the periphery of the placenta.

Acknowledgment
LOK was generously funded by an Early Career Grant and HDM by a Lab Visit Grant from the Society for Endocrinology.

P268
In support of the ‘Rotterdam’ PCOS criteria: Identical LH responses to GnRH stimulation in women with oligo-/amenorrhoea and polycystic ovaries regardless of androgen status
Krzysztof Lewandowski1, Agata Cajdler-Luba1, Małgorzata Bienkiewicz1 & Andrzej Lewinski1
1Department of Endocrinology and Metabolic Diseases, Medical University of Lodz, Lodz, Poland; 2Department of Endocrinology and Metabolic Diseases, Polish Mother, Memorial Research Institute, Lodz, Poland; 3Department of Quality Control and Radiological Protection, Medical University of Lodz, Lodz, Poland.

Objective
There is no universal consensus as to the criteria of PCOS (i.e. Rotterdam ESHRE/ASRM versus Androgen Excess Society (AES) criteria). Furthermore, androgen measurements within the female range are fraught with several methodological problems. As GnRH stimulation can reveal a relative LH excess, caused by an increased frequency of hypothalamic GnRH pulses, then we have endeavoured to assess gonadotrophin response to GnRH in women with PCOS and healthy controls.

Design, patients and methods
The study involved 155 subjects: PCOS (‘Rotterdam’ criteria), n = 121, age (mean ± s.d.) 24.8 ± 5.4 years, BMI 24.5 ± 6.0 kg/m², all with oligo-/amenorrhoea and polycystic ovarian morphology, and 34 regularly menstruating controls, matched for age and BMI, with normal ovarian morphology. Total testosterone, androstenedione, DHEAS, 17OH-progesterone and prolactin were measured in early follicular phase. LH and FSH were measured before and at 30 min post GnRH, 5.2 IU/l, at 0, 30 and 60 min. post GnRH, respectively, (P < 0.0001). An LH/FSH ratio in PCOS group increased further after GnRH stimulation (P < 0.01). ROC analysis revealed that LH 30min/FSH30min > 6.68 IU/l, 33.86 at 0, 30 and 60 min. post GnRH, respectively, (P < 0.0001). LH pulsatility within each subject was compared between the 4 h pre-injection and LH/FSH ratio than controls (P < 0.001).

Conclusions
Regardless of their androgen status, women with polycystic ovarian morphology and oligo-/amenorrhoea have higher baseline and GnRH-stimulated LH concentrations and higher GnRH-stimulated LH/FSH ratio in comparison to healthy controls. This suggests the existence of similar underlying mechanism accounting for menstrual irregularities. In our opinion these observations support clinical validity of diagnostic criteria for PCOS based on the Rotterdam consensus.

P269
Kisspeptin-54 injection stimulates activity of the human GnRH pulse generator in healthy women
Channa N Jayasena1, Alexander N Cominons1, Shiviha Misra1, Abbara Ali1, Tavare Aniket1, Mandy Donaldson2, Mohammed A Ghaetel1, Stephen R Bloom3 & Waljit S Dhillo1
1Section of Investigative Medicine, Imperial College London, Hammersmith Hospital, London, UK; 2Department of Clinical Biochemistry, Charing Cross Hospital, Imperial College Healthcare NHS Trust, London, UK.

Background
Kisspeptin is a novel hypothalamic hormone with powerful stimulatory effects on the hypothalamo-pituitary–gonadal (HPG) axis. Inactivating mutations in the kisspeptin receptor lead to pubertal failure. We have previously demonstrated that injection of kisspeptin-54 stimulates LH release in healthy men and women. Recent studies in animals suggest that endogenous kisspeptin may be involved in stimulating the GnRH pulse generator. Determining whether exogenous administration of kisspeptin can stimulate the human GnRH pulse generator, has important therapeutic implications.

Aim
To determine if kisspeptin-54 administration can stimulate the GnRH pulse generator in healthy female volunteers.

Methods
Six healthy female volunteers underwent frequent blood sampling for serum LH measurement every 10 min for 8 h (as a surrogate marker of the GnRH pulse generator) during the follicular phase of menstrual cycle. A s.c. injection of saline or kisspeptin-54 (0.3–0.6 nmol/kg) was administered 4 h after commencing the study. LH pulsatility within each subject was compared between the 4 h pre-injection and 4 h post-injection.

Results
No significant differences in number of LH pulses and LH pulse amplitude were observed before and after saline injection. A 6-fold increase in mean number of LH pulses was observed following kisspeptin-54 injection when compared the pre-injection period (mean number of LH pulses during 4 h: 0.50 ± 0.48, pre-injection; 3.00 ± 0.69, post-injection, P < 0.05 versus pre-injection). The LH pulse amplitude was nearly 2-fold higher after kisspeptin-54 injection when compared with the pre-injection period (mean pulse amplitude in IU/l during 4 h: 1.31 ± 0.09, pre-injection; 2.31 ± 0.25, post-injection, P < 0.05 versus pre-injection).

Conclusions
We have demonstrated for the first time that kisspeptin-54 stimulates activity of the GnRH pulse generator in humans. A single injection of kisspeptin-54 injection increases LH pulse frequency and LH pulse amplitude in healthy women. This data has important therapeutic implications for the future development of kisspeptin to treat patients with disorders of reproduction.

P270
Expression and functional activity of thyroid hormone transporters in microvillus plasma membranes from human term placental syncytiotrophoblasts
Laurence Loubiere, Elissavet Vasiliopoulou, Jayne Franklyn, Mark Kilby & Shiao Chan
University of Birmingham, Birmingham, West Midlands, UK.

Background
Thyroid hormones (TH) are vital for fetal and placental development. TH transporters including monocarboxylate transporters 8 and 10 (MCT8, MCT10), organic anion transporters (OATP1A2, OATP4A1) and system-L amino acid transporters (LAT1, LAT2) are expressed in human placenta from 6 weeks of gestation. All of these TH transporters have been localized to the human syncytiotrophoblast layer of placental villi, which is in direct contact with maternal blood. Thyroxine (T4) is postulated to be the major TH transported from maternal blood. T4 is transported into syncytiotrophoblasts via organic anion transporters (OATP1A2, OATP4A1) and system-L amino acid transporters (LAT1, LAT2) and enters the syncytiotrophoblasts. 

Methods and results
Maternal-facing syncytiotrophoblast microvillus plasma membranes (MVM) were isolated from normal human term placentae by MgCl2 precipitation and differential centrifugation. MCT10, MCT8, OATP1A2, OATP4A1 and LAT1 protein were detected in MVM using western-blot. [125I]T4 uptake by MVM vesicles was linear over the first 2 min and reached equilibrium after 20 min.
T₄ uptake was Na⁺-independent. Excess T₄ reduced [$^{125}$I]T₄ uptake by 22% (n=5, P<0.05). T₃ did not affect [$^{125}$I]T₃ uptake whereas TRIAC significantly reduced [$^{125}$I]T₄ uptake by 19% (n=3, P<0.01). Competitive inhibitors of TH transport by MCT8, MCT10 and LAT1, such as chlorpromazine, leucophosphate and bromosulphthalein, alone, did not affect [$^{125}$I]T₄ uptake. However, combinations of these inhibitors could significantly affect [$^{125}$I]T₄ uptake. The strongest inhibition of T₄ uptake was achieved with desipramine, a non-competitive inhibitor of TH transport mediated by MCT8, MCT10 and LAT1 (31%, n=4, P<0.05).

Conclusion

In human term placenta, TH transporters are ubiquitously present in syncytiotrophoblast MVM, which can regulate placental TH entry and transplacental TH transfer from the mother to the fetus. Our data suggest that maternal T₄ uptake by syncytiotrophoblasts is not mediated by a single predominant TH transporter but rather by a combination of these proteins.

---

**P271**

Increased epididymal Rnase9 expression in Rnase10 (−/−) mice

Victoria Sharp, Anton Krutsikkh, Jean-Louis Dacheux, Matti Poutanen & Ilpo Huhtaniemi

1Imperial College London, London, UK; 2UMRINRA-CNRS6175, Nozilly, France; 3University of Turku, Turku, Finland.

Spermatozoa are dependent on the proximal epididymis environment to complete their maturation. However, no single specific factor crucial for this process has been identified. Rnase9 and Rnase10 are expressed specifically in the murine proximal epididymis. Located on chromosome 14C1 only 28 kb apart, they do not show high homology with each other. An orthologue of both genes exist in the rat and human, and ‘Train A’ (porcine orthologue) is the most abundant (80–90%) secretory protein in the initial segment (IS). Testosterone replacement experiments revealed that Rnase9 expression is under direct/indirect regulation by androgens and Rnase10 expression is regulated by testicular factors other than androgen. We recently observed that spermatozoa from Rnase10 KO mice lacked association with gluttonous extra-cellular material in the distal epididymis, were released as single vigorously motile cells into bicarbonate-free medium, displayed no tendency for head-to-head agglutination and lacked affinity to the oviductal epithelium. Failure to gain the site of fertilization was associated with a gradual loss of ADAM3 protein from sperm surface during epididymal transit. Spermatozoa from KO mice are also unable to establish tenacious associations with the zona pellucida, yet they were capable of fertilization in vitro. The murine epididymal secretome of Rnase10 was assessed by incubating isolated IS fragments, with [$^{35}$S]-methionine. Accumulated methionine-labeled proteins were fractionated by 2D gel. In contrast to porcine Train A, murine Rnase10 secretion represents only 1.5–1.7% of the total IS secretion. Total RNA from IS of WT and Rnase10 KO mice was analysed using the Affymetrix GeneChip® Mouse Exon 1.0 ST Array. Rnase9 expression was increased 4.10 fold (P<0.05) as a result of the loss of Rnase10. Human Rnase9 is a sperm binding protein, whereas Train A does not interact directly with rabbit sperm. Therefore, Rnase9 may be an intermediary, involved in the stabilisation of ADAM3 to the sperm surface.

---

**P272**

Is age of vaginal opening an indicator of leptin sensitivity in female Sprague Dawley rats?

Michelle Sleeth, Kylie Beale, Emily Thompson, Jordan Baxter, Stephen Bloom & Kevin Murphy

Imperial College, London, UK.

Leptin is an adipocyte-derived cytokine critical to the integration of energy homeostasis and reproduction which putatively acts as a metabolic gate for the onset of puberty. However, it is unknown whether the age of puberty reflects leptin levels or leptin sensitivity. If pubertal age reflects leptin sensitivity, it may also reflect susceptibility to diet-induced obesity. We hypothesised that age of pubertal onset reflects leptin sensitivity. We assessed leptin sensitivity in female Sprague Dawley rats at three pubertal stages: pre-pubertal, peri-pubertal and one month post pubertal. Rats were administered an intraperitoneal injection of vehicle or leptin (3 mg/kg) at the onset of the dark phase in a cross-over design. Leptin sensitivity was assessed by measuring food intake following leptin versus vehicle at 4 and 24 h post-injection. Rats were retrospectively classified into early (days 33–36) or late (days 41–44) vaginal opening. Following vaginal opening, rats were switched to a high-fat diet (32% energy from fat) for 4 weeks and body weight monitored daily to determine whether age of pubertal onset predicted susceptibility to diet-induced obesity. Following 28 days of post-pubertal high-fat feeding, females who had undergone early vaginal opening remained significantly more sensitive to the anorexigenic effects of exogenous leptin than those which had undergone late vaginal opening. There was no significant difference detected in leptin sensitivity between rats which underwent early vaginal opening and those that underwent late vaginal opening in either the pre-pubertal or peri-pubertal period. There was no significant difference in overall body weight or food intake between the early and late groups at the end of the study. These data suggest that early vaginal opening may be associated with improved leptin sensitivity in later life, but that vaginal opening age does not predict acute leptin sensitivity in the pre-pubertal or post-pubertal period.

---

**P273**

A case of severe refractory hypercalcaemia in pregnancy caused by hypersecretion of parathyroid hormone related peptide (PTHrP) by the placenta.

Allison Martin & Mark Spring

Kingston Hospital, Surrey, UK.

A 39-year-old woman presented at 23 weeks gestation with extreme fatigue and non-specific neurological symptoms. Other than mild physical examination was normal. Serum calcium was 3.36 mmol/l (normal 2.12–2.62) and phosphate 0.8 mmol/l (Normal 0.8–1.5). She was severely hypercalcaemic throughout pregnancy and her corrected calcium ranged between 2.82 and 3.48 mmol/l. Other investigations included 25 hydroxy-Vitamin D 98 (normal 75–200), undetectable PTH <1.2 pmol/l (normal 1.6–6.9) confirmed by two laboratories, 1.25 dihydroxy-Vitamin D 209 pmol/l (normal value for pregnancy) and an inappropriately normal PTH related peptide level of 1.3 pmol/l (normal range 0.7–1.8 pmol/l). The serum angiotensin-converting enzyme and serum protein electrophoresis were normal.

A trial of steroids and intravenous fluids did not affect the hypercalcaemia. In view of poorly controlled hypertension and proteinuria she had caesarean delivery at 36 weeks. Both she and her male neonate were hypercalcemic at delivery but this fully resolved within 24 h, suggesting a placental aetiology of the hypercalcaemia. Regrettably the placenta was inadvertently discarded after delivery.

The literature suggests that the physiological changes in placental and mammary PTH related peptide production observed in pregnancy and the peripartum period might play a significant role in calcium homeostasis in both the mother and foetus. This may be independent of other calcitrophic hormones including parathyroid hormone and calcitriol. Parathyroid hormone related peptide hypersecretion in pregnancy is a rare entity and this has only very rarely been described. We believe this severe form of hypercalcaemia in both the mother and neonate was precipitated by an overproduction of PTH related peptide by the placenta. This case demonstrates the diagnostic and therapeutic challenges posed by this very rare but potentially life-threatening pregnancy related condition.

---

**P274**

Comparison of cardiovascular markers and variability of insulin resistance in women with anovulatory and ovulatory polycystic ovary syndrome

Li Wei Cho, Eric Kilpatrick, Brian Keavil, Vijay Jayagopal, Anne Marie Coady & Stephen Atkin

1University of Hull, Hull, UK; 2Changi General Hospital, Singapore, Singapore; 3Wythenshawe Hospital, Manchester, UK; 4York Hospital, York, UK; 5Hull Royal Infirmary, Hull, UK.

Objective

We aim to study whether a difference exists in cardiovascular risk and variability of insulin resistance in women with PCOS who are ovulatory and anovulatory. Method

Fifty-three women with PCOS were recruited. All subjects were diagnosed to have PCOS by the ESHRE/ASRM Rotterdam criteria where all patients had evidence of biochemical hyperandrogenism and hirsutism, chronic anovulation and polycystic ovaries on transvaginal ultrasound. Non classical 21-hydroxylase
P275
Male germ cell activity during perinatal reproductive development in the mouse
Shea Jarvis1, Robert Winston1, Scott Fraser2 & Carol Readhead2
1Imperial College London, Hammersmith Hospital, DU Canoe Road, UK;
2California Institute of Technology, E California Boulevard, Pasadena, California, USA.

Dynamic changes in gene expression patterns and cell behaviour are evident throughout embryonic and neonatal germ cell development in the mouse. In the testes, the postnatal period represents a time when the male germline stem cells (GSCs) or, gonocytes migrate to the basement membrane of the seminiferous tubules preparing for a lifetime of spermatogenesis and is an important area of study. Here we use the transgenic mouse that expresses green fluorescent protein under the Oct 4 promoter (Oct4::GFP transgenic mouse). A novel ex vivo model was developed to image cell migration, proliferation and apoptotic events during GSCs development in the seminiferous tubules of the late mouse embryo and neonate. In the male neonatal testes, the germ cells demonstrated motile behaviour as they translocate from the centre to the basement membrane of the seminiferous tubules. We identified live germ cell movements, proliferation and apoptotic events within the seminiferous tubules during the late fetal and early neonatal period in the mouse. Timelapse microscopy captured 2 peaks of apoptosis in the Oct4::GFP germ cells- during late embryonic stages and most notably at postnatal day 4 (P4), which was correlated with high levels of cleaved caspase-3 expression. At postnatal P4 there was terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) staining. Correspondingly, the number of Oct4::GFP cells per semiferous tubule (mean ± s.d.) decreased from 9.1 ± 3.67 cells/tubule at E17.5 to 2.7 ± 2.72 cells at P0, and 2.2 ± 1.89 cells by P5 thus correlating with apoptosis captured during live imaging. Concomitant germ cell proliferation was noted during apoptotic waves. Confocal videomicroscopy has allowed us to visualize live peri- and neonatal events during mouse testicular development. Male germ cells change their characteristics dramatically during perinatal development and the paracrine factors involved in this process is an exciting area of future work.

P277
Increased intensity of P450c17 protein expression in theca cells from polycystic ovaries
Fabio Comini, Kate Hardy & Stephen Franks
Imperial College London, London, UK.

Background/aims
Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women, being characterized by ovarian hyperandrogenism attributed to intrinsic overproduction by theca cells (TCs). Although previous reports pointed a higher stability and transcription of CYP17mRNA (Wickenheisser JK et al. 2006)1, few have examined (and none has quantified) P450c17 protein expression in TCs from PCOS ovaries. Therefore, the aim of this study is to quantify and compare protein expression of the steroidogenic enzymes in normal and PCOS ovaries employing the use of optical density measurements.

Materials and methods
We used quantitative immunohistochemistry (IHC) to examine expression of P450c17 and 3ßHSD in archived ovarian tissue from PCOS (n = 16) and normal women (n = 10) obtained from the Histopathology Bank of St Mary’s Hospital, London. Overall, 82 follicles were classified as healthy (n = 30) or atretic (n = 52) based on previously established criteria (Brailly et al 1981)2. Optical density measurements in theca cells were made using the software NIS-Elements AR 3.1(Nikon). Differences between groups were analysed using the Student t-test or Mann-Whitney test according with the distribution of data.

Results
Compared with normal ovaries, healthy follicles from PCOS had a higher proportion of theca cells strongly expressing P450c17 protein; PCOS: 0.40 (0.27-0.68) vs controls: 0.17 (0.07-0.37), (median, interquartile range); P = 0.04, Mann Whitney test). Intensity of expression, (optical density arbitrary units) was also increased in PCOS: 0.82 (0.52-1.2) vs controls: 0.57 (0.28-0.57), P = 0.04, (Mann Whitney test). No differences were seen in theca cell labelling for 3ßHSD protein in terms of its optical density; PCOS (mean ± s.d.) 0.16 ± 0.008 vs controls 0.13 ± 0.017, P = 0.19 (Student’s t-test).

Conclusion
The increased proportion of P450c17 positive cells and the higher intensity of staining support the view that dysregulation of 17-hydroxylase/17-20 lyase activity in theca is a key abnormality in the aetiology of hyperandrogenism in PCOS.

References

Acknowledgements
MRC, Capes Foundation, Dr R Parker (Univ Alabama,USA) (P450c17 Ab), Dr J L Mason (University of Edinburgh, UK) (3ßHSDII Ab).

P276
Defining insulin signalling pathways in granulosa-lutein (GL) cells in women with polycystic ovary syndrome (PCOS)
Jalini Joharatnam1, Giuseppina Lamanna2, Geoffrey Trew2, Stuart Laverty2, Kate Hardy1 & Stephen Franks1
1Imperial College, London, UK; 2Division of Reproductive Medicine, Imperial College Healthcare, NHS Trust, London, UK.

Aim
PCOS is associated with peripheral insulin resistance and we have previously shown that glucose uptake and metabolism are impaired in granulosa lutein (GL) cells of anovulatory women with PCOS (anovPCO). The aim of this study was to delineate insulin-signalling pathways in GL cells from women with anovPCO and to compare the response to insulin with that in GL cells from ovulatory women with (ovPCO) and without polycystic ovaries (controls).

Methods
Primary culture of GL cells obtained at time of egg collection from 3 groups of patients undergoing routine IVF: women with normal ovaries and regular cycles (n = 12), women with polycystic ovaries and regular cycles (n = 8) and women with polycystic ovaries and oligoamenorrhea (n = 11). Primary cultures of GL cells were incubated with insulin (1, 10, 100 mg/ml) and analysed for glucose uptake, lactate accumulation and progesterone production. Cell lysates were prepared for identification of insulin signalling pathways, specifically those involving P3 kinase (P3-K) and MAPK, using Western immunoblotting.

Results
As previously shown by there was impairment of glucose metabolism in cells from anovPCO women with conservation of insulin stimulated progesterone formation. Using phospho-specific antibodies to Akt Ser 473 and GSK 3ß Ser 9, no significant impairment insulin stimulated P3K signing was observed. In the MAPK pathways, p42 ERK phosphorylation was significantly greater in controls compared to anovulatory and ovulatory PCOS patients in response to 100ng/ml insulin.

Conclusions
We have confirmed selective resistance to insulin in glucose metabolism in granulosa lutein cells in women with anovulatory PCOS. The absence of any obvious impairment in the insulin-stimulated P3K signalling pathway in response to insulin in women with anovulatory PCOS is unexpected. We did however see impairment in MARK signalling and the significance of this finding with respect to impaired glucose metabolism in granulosa cells remains to be determined.
**P278**

**Neuromedin B stimulates the hypothalamo-pituitary–gonadal axis in male rats**

Charlotte Boughton, Sejal Patel, Anjali Amin, Emily Thompson, Mohammad Ghatei, Stephen Bloom & Kevin Murphy
Imperial College, London, UK.

Neuromedin B (NMB) is a highly conserved bombesin-related peptide found in mammals. The mammalian bombesin family of receptors consists of three closely related G protein coupled receptors, BB₁, BB₂ and BB₃. The BB₁ receptor subtype has the highest affinity for NMB. NMB mRNA is detected in the CNS and is expressed at relatively high levels in the rat hypothalamus, in particular the medial preoptic area and the arcuate nucleus.

NMB has well documented roles in the regulation of the thyroid axis and the stress axis in rats. However, there is little available data regarding the role of NMB in the regulation of the hypothalamo-pituitary–gonadal (HPG) axis. It is known that the NMB receptor is expressed in immortalised GnRH-releasing GT1-7 cells, and that anterior pituitary NMB-immunoreactivity is altered by changes in the sex steroid environment. The objective of these studies was thus to further investigate the effects of NMB on the HPG axis.

I.c.v. administration of NMB (10 nmol) to adult male rats significantly increased plasma LH levels 30 min after injection (plasma LH ng/ml; saline 0.7 ±0.1, 10 nmol NMB 1.3 ±0.2, P < 0.01). In vitro, NMB stimulated GnRH release from hypothalamic explants from male rats and from GT1-7 cells. NMB had no significant effect on LH release from anterior pituitary explants from male rats, or from LIT2 cells in vitro.

These results suggest a previously unreported role for NMB in the stimulation of the HPG axis via hypothalamic GnRH. Further work is now required to determine the receptor mediating the effects of NMB on the reproductive axis and the physiological role of NMB in reproduction.

---

**P279**

**Nesfatin stimulates the hypothalamic-pituitary–gonadal axis in male rats**

Michael Patterson, Katie Wynne, Sejal Patel, Keisuke Suzuki, John Tadross, Mohammad Ghatei & Stephen Bloom
Imperial College London, London, UK.

Nesfatin is an 82 amino acid peptide identified as a novel hypothalamic regulator of feeding. In rodents, central administration of nesfatin acutely inhibits feeding and chronic administration reduces weight gain. Subsequent research has demonstrated nesfatin is involved in the control of puberty in female rats. During puberty i.c.v. administration of nesfatin stimulates release of LH and FSH but has no effect in adult female rats.

We investigated the effects of i.c.v. injection of nesfatin on the release of pituitary hormones in ad libitum fed unanaesthetised adult male rats. In contrast to the data from adult female rats, i.c.v. administration of nesfatin (1 nmol) significantly increased plasma LH and FSH levels 30 min post injection. There were no significant changes in plasma ACTH, TSH and prolactin. In a second experiment, i.c.v. administration of nesfatin (1 nmol) significantly increased plasma testosterone 60 min post injection. To further investigate the role of nesfatin in the hypothalamic-pituitary–gonadal (HPG) axis we examined the effect of nesfatin on the release of GnRH from static hypothalamic explants. Treatment with 100 and 1,000 nm nesfatin significantly increased GnRH release from in vitro hypothalamic explants. Data from these studies suggests nesfatin is a novel regulator of the HPG axis in adult male rats.

---

**P280**

**Study of role of retinol-binding protein 4 in women with polycystic ovary syndrome**

Abdel-Sattar Elieib, Khalid Makboul & Rania Abd El Baki
Ain Shams University, Cairo, Egypt.

Retinol-binding protein 4 (RBP4) is a central mediator of obesity-induced insulin resistance in mice and humans. In addition, elevated serum RBP4 levels have been associated with cardiovascular risk factors and metabolic syndromes.

**Aim**

To investigate whether serum RBP4 level is correlated with metabolic parameters, indices of insulin resistance, and endocrine variables in PCOS women.

**Subjects and methods**

Our study was conducted on 90 women their age ranged from 20 to 39 years. They were divided into Group I: 30 lean patients with PCOS, Group II: 30 obese patients with PCOS, Group III: 15 lean control women and Group IV: 15 obese control women excluding those receiving oral contraceptives, glucocorticoids, oral hypoglycemic, anti-obesity or anti-androgenic drugs. Fasting and 2 h postprandial plasma glucose (2 h PPPG), HbA1c, fasting insulin level, HOME IR, lipid profile, LH, free testosterone, serum RBP4, serum uric acid, pelvic ultrasonography were done for all subjects.

**Results**

We found that serum RBP4, fasting insulin level and HOME IR were higher in PCOS group than control group. Furthermore, there was a high significant positive correlation between RBP4 and weight, BMI, waist circumference, WH ratio, fasting insulin, HOME-IR, triglyceride and LDL-c with P value <0.01, and a positive significant correlation between RBP4 and 2 h PPPG and HbA1c with P value <0.05 but there was a high significant negative correlation between RBP4 and HDL-c with P value <0.01.

**Conclusion**

Serum RBP4 is higher in the PCOS group than non-PCOS group, so RBP4 may play a role in the pathophysiology of PCOS. Further studies are needed to clarify the role of RBP4 in these women.

---

**P281**

**HRPE773 (ZG16B) expression is elevated in human endometrium during the early secretory phase of the menstrual cycle and in uterine decidua following miscarriage**

Bonnie Ng¹, Sarah McDonald¹, Xia Ren¹, John Mullins², Michael Rae³, Hillary Critchley¹, Andrew Hornie¹ & Steven Morley³
¹Centre for Reproductive Biology, University of Edinburgh, Edinburgh, UK; ²Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK; ³School of Life, Sport and Social Sciences, Edinburgh Napier University, Edinburgh, UK.

**Introduction**

Expression of murine CSP-1/Dcpp secretory proteins was first identified in sublingual salivary glands and subsequently in secretory epithelia of several other tissues, including the mouse female reproductive tract where expression is regulated by oestrogen. Preliminary studies indicated that HRPE773, the human CSP-1/Dcpp orthologue, displayed a similar pattern of expression to its murine counterparts. We therefore hypothesized that HRPE773 might be expressed in human endometrium and uterine decidua under the influence of steroid hormones.

**Methods**

Timed endometrial biopsies were collected with informed consent from patients undergoing elective hysterectomy or investigation for benign gynaecological conditions and during spontaneous miscarriage or termination of pregnancy. Quantitative Real-Time PCR and immunohistochemistry were used to determine HRPE773 mRNA levels and protein localization. HRPE773 expression was also measured in telomerase-immortalized human endometrial epithelial cells (hTERT-EECs) treated with oestradiol, medroxyprogesterone acetate (MPA), or both oestradiol and MPA for 8 and 24 h at physiological doses. Statistical analyses were performed using the Kruskal–Wallis test (CI 95%) with appropriate post hoc testing.

**Results**

HRPE773 was expressed in human endometrium in all phases of the menstrual cycle, but was significantly higher in the early-secretory phase compared to proliferative, mid-secretory, late-secretory or menstrual phases, while decidual expression was elevated in women with miscarriage, but not in extra-uterine (tubal) or viable intrauterine termination of pregnancy. HRPE773 immunoreactivity was located primarily in the cytoplasm of endometrial luminal and glandular epithelia, with some staining also in stromal cells. HRPE773 mRNA levels in hTERT-EECs treated with oestradiol alone and both oestradiol and MPA was significantly lower after 24 h compared to vehicle.

**Conclusion**

HRPE773 expression is elevated in endometrial luminal and glandular epithelia during the early secretory phase of the menstrual cycle and in uterine decidua following miscarriage, while cell culture experiments suggest that HRPE773 expression is likely to be inhibited by oestrogen.
P282

Case-control study of 5-alpha reductase type 2 Val89Leu polymorphism in Romanian PCOS patients
Serban Radian1,2, Cristina Ramona Radulescu1, Daniela Aforesi2, Monica Gheorghiu2, Nicoleta Buculescu1, Alice Albu1, Simona Fica1,3, Florin Grigorescu1 & Mihai Cociulescu1
1C. Davila University of Medicine, Bucharest, Romania; 2C.I. Parhon Institute of Endocrinology, Bucharest, Romania; 3Elias Emergency University Hospital, Bucharest, Romania; 4Molecular Endocrinology Laboratory, UMR-204 NUTRIPASS, Montpellier, France.

Background
Hyperandrogenism (HA) is required for diagnosis of polycystic ovary syndrome (PCOS) and it is central to PCOS pathogenesis. Putative mechanisms of hyperandrogenism include disregulation of steroidogenesis, plasma transport and peripheral tissue regulation. Therefore, 5-alpha reductase genes (SRD5A1, SRD5A2), that regulate peripheral tissue conversion of testosterone to more potent dihydrotestosterone are good PCOS candidate genes. The single published study of SRD5A1 and SRD5A5 in PCOS showed association of several SNPs haplotypes with PCOS, including rs523349 (Val89Leu in SRD5A2).

Aim
To test association of rs523349 with PCOS and related traits in the Romanian population.

Patients and methods
Two hundred and fifteen PCOS patients (Rotterdam criteria) and 107 eumenorrheic, non-hirsute controls, 15–40 years. old, recruited at the C.I. Parhon National Institute of Endocrinology and Elias Emergency University Hospital, Bucharest. rs523349 genotyping was performed by HRM-PCR (Corbett Research), with Razer probes (Primer Design, UK) and validated by direct sequencing.

Results
rs523349 allele frequency did not differ significantly between PCOS and control (0.72 vs 0.71, P=NS) and was comparable to that observed by Goodarzi et al. rs523349 genotypes were not significantly associated with PCOS. rs523349 (genotypes or allelic frequency) was not associated with PCOS phenotype/ biochemical traits. The statistical power of our study was limited (0.39), but comparable to the original study (0.5).

Conclusions
Our negative association result suggests that SRD5A2, through rs523349, is not a major gene in PCOS genetics in our population. However, exclusion of a true effect will require an adequately-powered study.

Steroids

P283

GP say they rely on endocrinologists to manage adrenal crisis and patient education for Addison’s disease
Katherine White & Alick Mackay
Addison’s Disease Self-Help Group, Guildford, UK.

In early 2010, the Addison’s Disease Self-Help Group sent an information pack to 10 500 GP practices across the UK, outlining the GP’s role in diagnosis and care of the Addison’s patient. We asked the practice head to return a reply-paid questionnaire, detailing the number of steroid-dependent patients in the practice, their repeat prescription length, if they had an in-date supply of injectable hydrocortisone and any comments on the challenges they faced in providing care for their steroid-dependent patients.

Predictably, only a tiny proportion of GP practices sent a reply (n=22), so that their responses do not offer statistical validity. Nevertheless, this small sample offers some qualitative insights.

The 22 GP practices that replied contained an average of 4.4 GPs, 6700 patients and 1.7 Addison’s patients. This is only a slightly higher proportion than epidemiological research would have suggested. Thirty-three percent of practices with steroid-dependent patients (n=18) reported that they were on 28-day repeat prescriptions. Only 3 of these practices were able to confirm that their patients had an in-date supply of injectable hydrocortisone. Several GPs stated that prevention and management of adrenal crisis was their biggest challenge, as neither practice staff nor their adrenal patients had a good understanding of what this entailed. Others said they had insufficient experience to offer guidance to the patient on what to do if they became unwell and relied on patients to ‘self-care’.

This small survey reinforces the importance of the endocrinologist’s role in providing patient education for the prevention and management of adrenal crisis.

P284

Dehydroepiandrosterone (DHEA) supplementation improves cognitive function in perimenopausal rhesus monkeys
Henryk Urbanski, Laurie Renner, Alison Weiss, Jamie Garten, Krystina Sorwell, Steven Kohama & Martha Neuringer
Oregon Health and Science University, Beaverton, Oregon, USA.

Age-related cognitive decline in postmenopausal women is thought to be partially related to the loss of sex steroids. Like women, old female rhesus monkeys (Macaca mulatta) undergo menopause and show an associated decline in circulating estradiol levels. Similarly, they show an age-related decline in the release of dehydroepiandrosterone (DHEA) from their adrenal glands. Because DHEA acts as a substrate for estradiol synthesis in the brain, it is plausible that DHEA supplementation could enhance cognitive function in the elderly. To test this hypothesis, old ovary-intact female rhesus monkeys were given 5 mg of oral DHEA each morning for 2 months; this administration paradigm was found to recreate the physiological 24-h circulating DHEA levels of young adults. In a delayed matching-to-sample test of recognition memory, with a 240-s retention interval, the regularly-cycling old animals showed a significant improvement in performance after 2 months of DHEA treatment, relative to age-matched irregularly-cycling perimenopausal monkeys (P<0.01, n=3 per group).

Irregularly-cycling animals showed no improvement relative to their baseline measurement. These findings demonstrate that 2 months of DHEA hormone supplementation is sufficient to cause a detectable improvement in cognitive function in old females. However, they emphasize that there is a critical period of sensitivity; for DHEA therapy to be effective, it needs to be initiated before the individuals show significant age-related attenuation of estradiol levels. Although the underlying mechanism is unclear, detection of 17p-HSD, 3b-HSD and aromatase enzyme gene expression in the prefrontal cortex and hippocampus, by RT-PCR, suggests that it may involve intracrine conversion of DHEA to estradiol within the CNS.

P285

Measurement of salivary testosterone in female samples using a highly sensitive LC–MS/MS assay
Brian Keay1, Philip McDonald1, Wendy McDowell2, Alan Wallace3 & Fred Wu4
1University Hospital of South Manchester, Manchester, UK; 2London School of Hygiene and Tropical Medicine, London, UK; 3Glasgow Royal Infirmary, Glasgow, UK; 4Manchester Royal Infirmary, Manchester, UK.

Methods
We have developed a highly sensitive LC–MS/MS assay in an attempt to improve the measurement of salivary testosterone in female samples.

A 200 µl saliva sample, calibrators or QC were mixed with 10 µl working internal standard (0.1 µg/l) and 1 ml of methyl tert-butyl ether (MTBE). Vortex mixed for 4 min and frozen at ~80°C (1 h). Unfrozen organic layer was transferred to a glass tube and evaporated. The residue was reconstructed with 100 µl of 50:50 mobile, vortex mixed and transferred to a 96-well microtitre plate. Liquid chromatography was performed using a Waters Acquity UPLC system. Extract (30 µl) was injected directly from the microtitre plate onto a C18 Acquity 1.8 µm HSS T3 analytical column (2.1×50 mm). A solvent gradient was used to elute testosterone and D5 testosterone from the column. TMS was performed on a Waters Quattro Xevo TQ-S in positive ionization mode. Multiple reaction monitoring transitions for testosterone were m/z 289.2 → 108.8 (quantifier) and m/z 289.2 → 196.8 (qualifier) and for D5-testosterone internal standard was m/z 294.1 → 99.8.

Results
Lower limit of quantitation was 2 pmol/l, recovery was 98–105%. Interbatch precision (CV) was 4.4, 6.0 and 2.8% at 17, 100 and 154 pmol/l respectively. The median female range (n=96) was 10–15 pmol/l and the median male range (n=96) was 100–150 pmol/l. Although cross validation of calibrators was
Steroid Replacement Education: Impact on Patients and their Carers
Mujahid Saeed, Theingi Aung, Judy MacDonald, John Wass & Niki Karavitaki
Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK.

Steroid replacement is life-saving in patients with steroid axis deficiency; education surrounding steroid replacement is vital in their management. We organised 'Steroid Replacement Education Days' for patients and their family members/carers to help enhance their knowledge on steroids and intercurrent illness. Lectures detailed manifestations, causes, management of steroid deficiency and the practical issues surrounding steroid replacement. This was followed by a practical demonstration of steroid emergency injections by our endocrine specialist nurse team. Questionnaires were offered to attendees to look at the impact of the course.

The patient questionnaire had 16 questions and there were 33 respondents. 51.5% (n = 17) reported that they had received prior training on adjusting the dose of steroids during intercurrent illness, with 45.5% (n = 15) having undergone adrenal crisis due to untimely adjustment of their steroids. 27.2% (n=9) were already fully confident (score rating of 8–10 on a 0–10 scale) in adjusting their steroids; this increased to 81.8% (n=27) after the training day. From 61% (n=2), 45.5% (n=15) felt fully confident in injecting steroids after the course. 94% (n=31) found the practical demonstration by the nurses extremely useful and a similar percentage rated the course excellent (score rating of 8–10 on a 0–10 scale). The questionnaire given to guests had 12 questions and 24 responded. 16.7% (n=4) had been trained on steroids and intercurrent illness prior to the course. 12.5% (n=3) felt confident in injecting steroids before; this rose to 50% (n=12) after the practical demonstration, which 87.5% (n=21) rated as excellent. 91.6% (n=22) rated the event as excellent.

Surprisingly, the confidence of patients on steroid replacement in dealing with intercurrent illness remains suboptimal. Events educating them and their carers are required on a regular basis aiming to enhance understanding of steroid treatment and to improve confidence in the emergency and life-saving management.

How reproducible are LC–MS testosterone results? A calibration exercise
Brian Keevil, Philip McDonald & Laura Owen
University Hospital of South Manchester, Manchester, UK.

Introduction
It has been recognised in EQA schemes in Europe and America that the reproducibility between labs using LC–MS for testosterone analysis is not optimal for this technique. We decided to conduct a calibration exercise to investigate the variability seen between labs.

Methods
Aqueous and matrix matched serum samples were sent to labs participating in the NEQAS testosterone scheme. The labs were asked to measure these samples blind using their routine assays and their own calibration material. We then re-calculated the results for these samples using assigned values from our routine assay which has been shown to align closely to a reference method. Most labs used liquid-liquid extraction to prepare samples but a variety of different LC columns and calibration matrices were used.

Results
Re-calculating the results from serum samples using matched serum calibrators improved the inter laboratory precision (CV) from 10% down to 5%, likewise re-calculating the results from aqueous samples using aqueous calibrators improved the inter laboratory precision from 7% down to 3%. Trying to re-calculate the serum results using aqueous calibrators actually made the inter laboratory variability worse. This suggests that the matrix in which the calibrator is made will affect the result significantly.

Conclusion
All laboratories gave clinically acceptable results but the accuracy of the results and hence the variability between laboratories was improved if common calibration material was used. If labs are to use a variety of different LC columns it is important that they thoroughly evaluate the effects of ion suppression, caused by sample matrix, which can vary profoundly with LC columns from different suppliers. The choice of column will also have a bearing on the type of calibrator matrix that is suitable for use in the assay.

Glucocorticoid receptor antagonism as a decision making tool in patients with adrenal incidentaloma and low-grade excess cortisol secretion: a pilot study
Miguel Debono, Sam Houghton, Richard Eastell, Richard Ross & John Newell-Price
Academic Unit of Endocrinology, University of Sheffield, Sheffield, South Yorkshire, UK.

Aims/hypothesis
Adrenal incidentaloma (AI) are very common, but optimal management of patients with AI and low-grade excess cortisol secretion is not established. Uncontrolled studies reporting outcomes of adrenalectomy suggest improvements
in cardiovascular risk, but all are subject to selection bias, and it is unclear if benefits are due to removal of excess cortisol. We reasoned that short-term use of mifepristone, a rapidly-acting glucocorticoid receptor (GR) antagonist, could improve GR-mediated cardiovascular and metabolic risk, with the ultimate aim of devising a individualised means of selecting those most likely to benefit from surgical intervention.

Methods

In a prospective open label pilot study, six patients with mild cortisol excess from adrenal incidentalomas (mean serum cortisol post 1 mg ONSD was 79.8 nmol/l) were treated with mifepristone 200 mg twice/day for up to 8 weeks. Primary endpoints were 2-h glucose from OGTT and resting/24-h BP at 4 weeks. Secondary endpoints included insulin sensitivity, fasting and AUC insulin and glucose at 4 weeks, resting/24-h BP at 8 weeks, serum 0900h ACTH/cortisol and salivary 0900/2300 h cortisol at 4 and 8 weeks, lipids and bone markers at 8 weeks.

Results

All subjects showed clear biochemical evidence of GR antagonism, with significant elevations of serum and salivary cortisol, and plasma ACTH. As a group, at 4 weeks, there was a significant improvement in all indices of insulin sensitivity/resistance including HOMA-IR (3.16 vs 2.3; P = 0.05), HOMA-%B (147.6 vs 91.67; P = 0.03) and Matsuda index (3.31 vs 4.98; P = 0.03). In one subject, however, there was no improvement. There were no significant changes in resting/24-h BP, mean serum osteocalcin levels and urine NTX/Creat.

Conclusions/interpretation

Short-term GR antagonism with mifepristone improves insulin sensitivity in some, but not all, patients with mild cortisol excess. Larger studies are needed, but our data suggest that GR antagonism may form the basis of a clinical tool to stratify patients for intervention in an individualised manner.

P291


tetrahydrodeoxycorticosterone excretion is a significant independent predictor of LV mass in patients with chronic kidney disease

Emily McQuarrie1, Patrick Mark1, Rajan Patel1, Robert Fraser1, Eleanor Davies1, Tracey Steedman2, John Connell2 & E Marie Freel1

1Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK; 2Cardiac MRI Unit, Western Infirmary, Glasgow, UK; 3Ninewells Hospital and Medical School, University of Dundee, Dundee, UK.

Aim

Increasing left ventricular mass index (LVMI) is associated with a poor prognosis in patients with chronic kidney disease (CKD). Blockade of the mineralocorticoid receptor (MR) in CKD leads to improvement in proteinuria and LV mass. However, the MR can be activated by aldosterone, cortisol and deoxycorticosterone (DOC) and their relative contribution to adverse events in CKD is unclear. We aimed to assess the correlation between mineralocorticoid excretion and LVMI in patients with CKD.

Methods

Fifty-eight patients with CKD stages 3/4 and 29 patients with essential hypertension (EH) were recruited. Patients underwent 24 h urine collection for urinary tetrahydrodeoxycorticosterone (THAldo) and tetrahydrodeoxycorticosterone (THDOC) (measured using GCMS), urinary electrolytes and protein (QP). LVMI was measured using gold standard cardiac MRI scanning. Factors which correlated significantly with LVMI were entered into linear regression models.

Results

Mean age was 57.1 years, 75.9% were male, mean BMI 29.2 kg/m², systolic blood pressure (SBP) 148 mmHg, DBP 86 mmHg and the mean number of anti-hypertensive agents was 2.4 (range 0–6). In CKD patients, mean eGFR was 38.5 ml/min per 1.73 m² and median QP 0.8 g/24 h. LVMI was significantly higher in patients with EH compared with CKD (61.6 ± 81.2 g/m²², P = 0.009). There were no significant differences in drug therapies, THAldo or THDOC excretion between the two groups. In patients with CKD, significant predictors of LVMI were male gender, SBP, 24-h QP, THAldo and THDOC excretion. On multivariate linear regression analysis, the significant independent predictors were SBP, male gender and THDOC excretion. THAldo was not an independent predictor. In patients with EH, plasma aldosterone concentration was the only significant independent predictor of LVMI. No association was seen with THAldo or THDOC.

Conclusions

Using cardiac MRI to assess LVMI, we have demonstrated that the mineralocorticoid THDOC is a novel independent predictor of LVMI in patients with CKD and not subjects with essential hypertension.

P292

Glycyrrhetinic acid disrupts the synthesis of adrenocorticosteroid hormones at multiple sites

Emad Al-Dujaili1, Christopher Kenyon2, Moira Nicoll2 & Ian Mason1

1Queen Margaret University, Edinburgh, Scotland, UK; 2QMRI, Edinburgh University, Edinburgh, Scotland, UK.

Background and aims

Previously we showed that the bioactive liquorice constituent glycyrrhetinic acid (GA) inhibits not only 11β-hydroxysteroid dehydrogenase enzymes but also the sulphation of corticosteroids by the enzyme sulphotransferase 2A1 (SULT2A1). Since pregnenolone, 17α-hydroxy-pregnenolone, deoxycorticosterone (DOC) and dehydroepiandrosterone (DHEA) are all prime substrates for SULT2A1, GA could have important effects in directing pathways of glucocorticoid, mineralocorticoid and androgen biosynthesis. This hypothesis has been investigated by comparing aldosterone, cortisol, cortisone, corticosterone, DOC, DHEA and testosterone output by human adrenocortical H295 cells treated with GA in the presence of added steroids.

Methods

Cells were incubated for 24 h in DMEM/F12, with 0, 3, 10, 25 or 100 μM GA and either 0.2 μM DOC or 1 μM DHEA. Free and conjugated steroids in the incubation medium were analysed by in-house ELISAs following extraction with Sep-Pak C18 cartridges and hydrolysis with Helix pomatia juice. SULT2A1 expression in cells was quantified by RTPCR.

Results and discussion

GA inhibited DHEA and DOC conjugation in a dose-dependent manner without affecting SULT2A1 transcription. However, total DOC (free + conjugated) was significantly increased in a dose-dependent manner as was glucocorticoid production. These results are consistent with transcript data which demonstrated that GA upregulated SULT2A1 expression.

Endocrine Abstracts (2011) Vol 25
negatively correlated with GA inhibition of sulphation in cells incubated in the presence of either DOC or DHEA \((r = -0.74, P < 0.02)\). We suggest that inhibition of pregnenolone sulphation (an early endogenous intermediate) channels steroidogenesis towards mineralocorticoid/glucoorticoid hormones. However, the total amounts of cortisol, cortisone and aldosterone were reduced rather than increased by GA; corticosterone levels were increased but not in line with DOC changes. Together, these results suggest that GA treatment affects the synthesis of 17α-hydroxylated steroid hormones \((> 75\% \text{ reduction in cortisol:}
\text{corticosterone ratios; } P < 0.01)\). DOC to aldosterone ratios were also reduced \((P < 0.001)\).

**Conclusions**

GA effects on steroid hormone profiles could be more complex than hitherto appreciated. It seems that GA can modulate adrenal steroidogenesis in vitro at multiple sites. The possibility of adrenocortical side-effects is, therefore, an important consideration in the development of 11β-HSD enzyme inhibitors to modulate glucoorticoid activity.

**P293**

Clinical outcomes of 250 μg short synacthen tests in a tertiary referral centre

Hiang Leng Tan, Srilatha Dampetla, Jen M Ng & Ammar Wakil

Diabetes Center, Michael White Center, Hull Royal Infirmary, Hull, England, UK.

**Introduction**

The short synacthen test (SST) is commonly used to assess the hypothalamic pituitary adrenal (HPA) axis in clinical practice. We evaluated the underlying clinical conditions pre-empting the request for a SST in a general medical ward and the adequacy of clinical follow up of patients with suboptimal responses to the test.

**Methods**

All patients with suboptimal SST results (30 min cortisol level 450-540 nmol/l) performed in the Acute Assessment Unit in our hospital between 01/10/2007 to 30/09/2009 inclusive were analysed. All patients who were on steroid therapy for more than 1 month or were deceased were excluded from the analysis.

**Results**

One hundred and twenty-two patients were included in the study. The different clinical indications for performing SST were as follows; recurrent falls/postural hypotension, 47 patients (38.5%), weight loss, 17 patients (13.9%), hypotension, 14 patients (11.5%), pre-post pituitary surgery, 13 patients (1.7%) and others, 31 patients (25.4%). Of the 122 patients with suboptimal SST, 98 patients received further clinical follow up; 44 of which were consequently treated with steroids.

**Conclusions**

The SST is a commonly used endocrinological test in clinical practice. The findings show that almost a fifth of patients tested with a SST may not receive any further follow up as evidenced by the absence of any documentation in the clinical case notes.

**P294**

Cortisol decreases lipogenesis in human hepatocytes

Maryam Nasiri, Laura Gathercole, David Hautoy, Stuart Morgan, Iwona Bajalska, Paul Stewart & Jeremy Tomlinson

The University of Birmingham, Birmingham, UK.

Glucocorticoid (GC) excess (Cushing’s syndrome) is characterized by central obesity, insulin resistance and in up to 20% of cases, non-alcoholic fatty liver disease (NAFLD). NAFLD is a progressive spectrum of disease ranging from hepatic steatosis to steatohepatitis, fibrosis and cirrhosis. Many processes contribute to lipid accumulation within hepatocytes including de novo lipogenesis which includes the rate-limiting carboxylation of acetyl CoA to malonyl-CoA by acetyl CoA carboxylase (ACC) and conversion to palmitate by fatty acid synthase (FAS). We have hypothesized that increased GC exposure drives insulin resistance and promotes the development of NAFLD.

C3A cells (human hepatoma derived), which express the GC receptor, were treated with cortisol (250 nM, 24 h) in the presence or absence of insulin (80 nM, 24 h). Lipogenic gene expression was determined by real-time PCR and lipogenesis measured by \(1^\text{14C}\) acetate incorporation into triglyceride. Cortisol increased mRNA expression of ACC2 (0.005 ± 0.003 (control) vs 0.008 ± 0.004 (cortisol), \(P < 0.05\)) and FAS (0.071 ± 0.007 (control) vs 0.110 ± 0.019 (cortisol), \(P < 0.05\)) but not ACC1, diacyl glycerol acyl transferase (DGAT) and glycerol phosphate acyl transferase (GPAT). However, cortisol decreased functional lipogenesis (78.68 ± 4.5% vs control (100%), \(P < 0.05\)) probably reflecting rate-limiting, post-transcriptional regulation. Interestingly, in the absence of cortisol, insulin only had a modest effect to increase lipogenesis and this did not reach statistical significance (114.9 ± 12.4% vs control (100%), \(P = \text{ns}\)). In contrast, when C3A cells were pre-treated with cortisol for 24 h, the stimulatory effect of insulin upon lipogenesis was augmented (mean % increase in lipogenesis following insulin, 14.9 ± 12.4% (control) vs 38.2 ± 12.3% (cortisol), \(P < 0.05\)).

In C3A cells, in the absence of insulin, GCs decreased lipogenesis despite increasing FAS and ACC2 expression. Consequent increased hepatic free fatty acid exposure may contribute to hepatic insulin resistance. Our data also suggest that GCs may potentially enhance the ability of insulin to promote lipid storage in hepatocytes.
Background

Human saliva is a valuable and flexible source of endocrine biomarkers, from which several significant steroids representing indices of development, well being, stress and reproduction can be quantified. Although for many disciplines blood represents the ‘gold standard’ for endocrine measurement, it also has its limitations, specifically requiring trained phlebotomists and appropriate facilities. The painful and invasive nature of blood draws can deter research participation and restricts frequent collection. Alternatively, saliva collection is straightforward in settings beyond clinical and laboratory boundaries and advances our access to populations and behavioural contexts for which blood sampling is unfeasible.

Objectives

This validation project investigates optimal protocols to adopt when collecting and storing saliva to improve reliability and increase comparability across research sites and studies. Our primary objective was to investigate and validate methods to successfully preserve saliva in a manner compatible with Enzyme Immuno-Assay (EIA). Identification of a preservation method could enhance flexibility of both field site conditions and collection protocols. This work is critical as previously established preservatives, such as sodium azide that were compatible with RIA, interfere with Horseradish Peroxidase commonly found in most EIA preparations.

Methods

Ethical Approval was granted by the local recognised University Ethics Committee. Raw human saliva samples were collected, spiked with different concentrations of the preservative Proclin300 and stored at room temperature. Following defined time periods (7d, 1, 3 and 6 months) samples were analysed for reproductive steroids using commercially available EIA kits, estradiol and progesterone.

Results and conclusions

Early results suggest that Proclin300 has potential for preserving saliva samples. Further novel preservation data are presented. We also report the stability of saliva content in conjunction with collection vial material (e.g. polystyrene, polypropylene). Such validation data are essential to further develop opportunities to capture endocrine profiles that are beyond reach of clinical settings.
It was concluded that the results of an SST always requires interpretation in view of the clinical scenario. Alteration of the reference range for cortisol would lead to an increased pass rate but might not change management greatly. The test should be repeated if the re is any doubt.

P300
Concurrent analysis of 10 serum steroids by mass spectrometry: investigation of the viability of the Perkin-Elmer CHS™ MSMS steroids kit on a waters xevo mass spectrometer with acuity UPLC system
Angela Taylor1, Cedric Shackleton1, Stuart Taylor2, Ulrich Glumer Jensen2, Marko Ojala2 & Wiebke Arlt3
1University of Birmingham, Birmingham, UK; 2Perkin-Elmer LAS (UK) Ltd, Buckinghamshire, UK.

Steroids present in human serum can be used to identify a number of conditions such as congenital adrenal hyperplasia, Addison’s disease and Cushing’s disorder. Historically this has been completed using immunoassays; currently there is a move towards mass spectrometry which reduces analysis time, cross reactivity and improves sensitivity. When mass spectrometers are coupled with liquid chromatography systems (LC-MS) it is possible to monitor a number of steroids in a single assay. Problems with inter-laboratory variation of LC-MS results can lead to errors when analysing nationwide data sets. Implication of a kit with its own mass spectrometry parameters such as sample preparation methods, columns, solvent additives and mass transitions should reduce inter-laboratory variation. Recent introduction of a 10 steroid serum kit (Perkin-Elmer) has been completed, due to the number of different mass spectrometers available these kits needs to be thoroughly tested on a number of systems to ensure comparability. The viability of the Perkin-Elmer 10 steroid CHS MSMS kit was examined on a Waters Xevo TQ mass spectrometer with an Acquity UPLC system. We carried out concurrent analysis over a range of concentrations for each corticoid: aldosterone (0.8–18 nmol/l), androstenedione (0.3–74 nmol/l) corticosterone (1–204 nmol/l), cortisol (5–1008 nmol/l), DHEA (1–255 nmol/l), DHEAS (37–7557 nmol/l), 11-deoxycorticisol (0.2–54 nmol/l), 17-hydroxyprogesterone (0.5–77 nmol/l), progesterone (0.5–91 nmol/l) and testosterone (0.1–26 nmol/l). The method was validated through investigation of spiked serum (Randox) and sera of healthy volunteers. A concurrent analysis of 10 steroids using LC-MS was completed with reliable quantitation in <15 min. For optimum results using a Xevo mass spectrometer a number of parameters were adjusted, for example the ESI source was superior to the APCl and some mass transitions were altered. The concurrent analysis of multiple steroids in a single LC-MS run demonstrates a major advance in the fast reliable diagnosis of steroid related disorders, which can be employed in clinical laboratories.

P301
An atypical case of familial glucocorticoid deficiency without pigmentation caused by coexistent homozygous mutations in MC2R (T152K) and MC1R (R160W)
Claire Hughes1, Setrap Turan2, Zeynep Atay3, Tulay Gurun2, Abdullah Bereket2, AdrianClark & Louise Metherell1
1Barts and the London School of Medicine, Centre for Endocrinology, WHRI, London, UK; 2Department of Paediatric Endocrinology, Marmara University, Istanbul, Turkey.

Familial glucocorticoid deficiency (FGD) is a rare autosomal recessive disorder characterised by unresponsiveness to ACTH and isolated cortisol deficiency. FGD is caused by mutations in genes encoding the ACTH receptor (melanocortin 2 receptor (MC2R)), its accessory protein (MRAP) or the steroidogenic acute regulatory protein (STAR). One significant feature is generalized skin hyperpigmentation which is thought to be due to elevated ACTH acting on the melanocortin 1 receptor (MC1R). MC1R plays a central role in the regulation of skin pigmentation, is expressed in melanocytes and binds α-MSH and ACTH with similar affinity. MC1R activation increases the ratio of black, strongly photoprotective eumelanin to reddish, poorly photoprotective phaeomelanin. Several MC1R variants are associated with red hair/fair skin.

The index case presented aged 4 years with hypoglycaemia after prolonged fasting during a respiratory tract infection. She had further hypoglycaemic attacks and was diagnosed with hypoglycaemia at 6 years (14 nmol/l) due to a genetic study of ACTH and hypercorticotropinemia (ACTH > 1250 pg/ml). Her parents were consanguineous and she had one unaffected sister. Her physical examination was normal except her height and weight were greater than the 97th centile for age. Interestingly, she had no hyperpigmentation despite very high ACTH levels. Nucleotide sequence analysis revealed homozygous mutations c.478C>T in MC1R and c.455C>A in MC2R leading to R160W and T152K changes in the proteins respectively. The R160W MC1R change has previously been implicated in a red hair/pale skin phenotype and the T152K change in MC2R is novel. Both parents were heterozygous for the mutations and her unaffected sister was heterozygous for the MC2R mutation and had a wild-type MC2R.

We report an unusual case of FGD without hyperpigmentation due to co-existent MC1R/MC2R mutations. This case is important as it clearly demonstrates for the first time that the assumption that skin pigmentation is caused by the action of ACTH on the MC1R is correct.

P302
Cushing’s syndrome in a patient with two lung tumours
Haliza Haniff, Andrew F Scarsbrook & Stephen M Orme
Leeds Teaching Hospitals NHS Trust, Leeds, UK.

A 21-year-old man presented with 2 months history of weight gain, acne, hirsutism, lethargy, and muscle weakness. Examination revealed that he was cushingoid in appearance, had pastular acne and proximal myopathy. Initial 24 h urinary free cortisol was significantly raised at 5320 nmol/day (10–147), with raised ACTH of 120 ng/l (<47). He failed to suppress his cortisol on the low dose dexamethasone suppression test (baseline 708 nmol/l and 48 h 706 nmol/l). Urinary and plasma 5-HIAA were normal. MRI pituitary showed no abnormality. He was started on metyrapone.

Thoracic CT scan revealed a 2 cm speculated soft tissue nodule in right upper lobe and 1 cm well defined, round nodule in the left lower lobe. Further imaging (octreotide scan & PET-FDG scan) revealed the nodules to be consistent with functioning neuroendocrine tumours, with the right upper lobe nodule being markedly more FDG-avid than the left. The adrenal glands were bulky consistent with ACTH-driven adrenal hyperplasia. The patient underwent a right upper lobectomy but histology revealed an unexpected finding- cryptococcal infection instead of a neuroendocrine tumour. He had never travelled abroad and was HIV negative. Post-operatively, his Cushing’s was still active and he was recommenced on metyrapone. He also received fluconazole for 6 months. He then underwent inferior petrosal sinus sampling (IPSS) which did not indicate a pituitary source of ACTH. He subsequently had a left lower lobectomy. Histology and immunohistochemistry confirmed a carcinoid tumour. Post-operatively, his MGMST showed a peak cortisol of <50 nmol/l indicating cure. Although there are a handful of cases describing opportunistic cryptococcal infection in pituitary Cushing’s disease, we believe that this is the first described case of opportunistic cryptococcal infection in a patient with Cushing’s syndrome due to bronchial carcinoid.

P303
Accidental long-term ingestion of androgenic steroid in a young female: a case report
Venkata Katreddy, Jane Dale & Haroon Siddique
Russells Hall Hospital, Dudley, Westmidlands, UK.

Aim
We present an unusual case of long-term accidental ingestion of androgenic steroid in a young female.

Case
A 28-year-old lady presented with male pattern of hair growth, weight gain of three stones, change in voice, and secondary amenorrhoea. On direct questioning she admitted she was taking ‘fat bursting pills’ for nearly 6 months, obtained from a gym, to lose weight. Examination revealed a blood pressure of 150/77 mmHg, increased muscle bulk, hirsutism and significant clitoromegaly.
Investigations

FSH 1.8 IU/l, LH 0.9 IU/l, oestradiol 169 pmol/l (8–2500), prolactin 268 mU/l (0–445), SHBG 16 nmol/l (18–114), androstenedione 9.7 nmol/l (1.0–11.5), 17 OH progesterone of 2.9 nmol/l (0.7–17.4) and testosterone of 29.2 nmol/l (0–2.8). Ultra sonogram showed normal ovaries, MRI revealed normal adrenal glands. The pills were stopped immediately. Within 4 months she had substantial weight loss, her periods returned, her physical appearance changed and blood pressure dropped to 116/80 mmHg. The testosterone levels dropped to 3.7 nmol/l and SHBG improved to 31 nmol/l.

Discussion

Androgenic steroid hormones are increasingly used by male and some female athletes to improve their performance. In one survey with 1667 participants 2.3% of women had taken an androgenic steroid at some point. Our case is unique as the ingestion was accidental and had resulted in physical changes. Change in voice, increased facial hair growth, climorogamely, decreased body fat, menstrual irregularities and aggressiveness are some of the perceived side effects. Chronic administration results in supraphysiological concentrations of testosterone and decreased SHBG as seen in our case. Stopping the pills resulted in reversal of these values. The reversal of clinical and biochemical abnormalities, confirms the diagnosis of exogenous androgenic steroid ingestion.

Conclusion

Accidental ingestion of long term androgenic steroid is uncommon. A clear history and a high level of suspicion are helpful in such circumstances, for the managing physician.

---

**P304**

Exogenous Cushing’s syndrome due to topical corticosteroid application

Ahmed Elsadig, Nisha Kaimal & Tara Kearney

Salford Royal Hospital, Manchester, UK.

Introduction

Prolonged use of topical corticosteroids causes systemic adverse effects including Cushing’s syndrome and hypothalamic–pituitary–adrenal (HPA) axis suppression, which is less common than that of the oral or parenteral route.

History

A 31-year-old female patient was referred from the dermatology clinic with symptoms and signs consistent with Cushing’s syndrome. She has been treated for psoriasis with a prolonged course of topical corticosteroids. She has been using the cream more often than has been advised by the treating dermatologist.

Clinical examination

Examination showed excess facial hair, moon-shaped face, centripetal obesity, purple striae on abdominal wall, an obvious buffalo hump and some areas of pigmentation in her skin. Systems examination revealed proximal myopathy.

Investigations and diagnosis

9 am cortisol was <10 nmol/l. Short synacthen test showed cortisol level of <10 nmol/l at 0 min, 29 nmol/l at 30 min. She was diagnosed with exogenous Cushing’s syndrome due to topical corticosteroid application.

---

**P305**

Is there a threshold morning cortisol level at which to perform the short synacthen test?

Sumudu Bujawansa, Shalini Kunasegaran, Steve McNulty, Kevin Hardy, Mohammad Al-jabouri, Niall Furlong & Upendram Srinivas-Shankar

St Helens and Knowsley Teaching Hospitals NHS Trust, St Helens, UK.

Introduction

Short synacthen test (SST) is of value in assessing the adequacy of hypothalamic–pituitary–adrenal axis (HPA). Although it is extensively used, it is unclear at what morning cortisol concentration one should consider performing the SST.

**Methods**

Retrospective observational study of consecutively performed SST (250 µg) between January 2009 and March 2010. Plasma cortisol was measured by enzyme immunoassay (Siemens Advia, Siemens plc, UK). A cut-off value of ≥550 nmol/l was used to differentiate adequate from inadequate response.

**Results**

76 consecutively reviewed endocrine out patients (females n (%) 53 (70)), median age (interquartile range) 46.5 (35.0–55.7) years underwent SST. Baseline and 30-min cortisol levels were 367 (266–502) and 745 (640–845) nmol/l respectively. Cortisol levels increased by 318 (219–436) nmol/l. There was no correlation between age and rise in cortisol level from baseline. There was no significant difference between baseline and 30 min cortisol and rise in cortisol between males and females. 50% (7/14) patients with baseline cortisol <250 nmol/l had 30 min cortisol <550 nmol/l. No patient with a baseline cortisol >250 nmol/l had 30 min cortisol <550 nmol/l (sensitivity 57%, specificity 91%), 92% (70/76) underwent the test before 1100 h. 30 min (not baseline) cortisol levels of the SST done after 1100 h versus before 1100 h were significantly lower, 751 (642–851) vs 642 (524–699); P = 0.04.

**Conclusion**

Morning cortisol levels of ≥250 nmol/l seems to be associated with adequate adrenocortical reserve. In such situations it may not be necessary to do a SST, unless clinical suspicion is high. Further studies are needed to determine the effect of timing of SST on its sensitivity and specificity.

---

**P306**

Why does MRAP2 fail to save familial glucocorticoid deficiency type 2 patients?

Rebecca Gorrigan, Leonardo Guasti, Adrian Clark & Li Chan

Barts and the London School of Medicine and Dentistry, London, UK.

Background and aims

The melanocortin-2-receptor accessory protein (MRAP) is essential for melanocortin-2-receptor (MC2R) function through receptor trafficking and signalling, enabling adrenal glucocorticoid synthesis in response to ACTH stimulation. Disabling mutations of MRAP result in life-threatening glucocorticoid deficiency, known as familial glucocorticoid deficiency type 2. MRAP has a single paralogue in the human genome, MRAP2. In vitro MRAP2 has a similar action to MRAP, facilitating MC2R function. However patients with FGD type 2 fail to be rescued by a functioning MRAP2 protein – the aim of this study is to investigate why.

**Methods**

cAMP production in response to ACTH stimulation of HEK293 cells transfected with MC2R and MRAP/MRAP2/MRAP and MRAP2 was quantified using dual-reporter luciferase assays. In situ hybridisation (ISH) was used to localise adrenal MRAP, MRAP2 and MC2R mRNA expression. MRAP and MRAP2 adrenal expression was quantified using real-time qPCR.

**Results**

Higher concentrations of ACTH were required to induce cAMP production in HEK293 cells transfected with MC2R/MRAP2 compared with cells transfected with MC2R/MRAP (EC50 9.3e-007 vs 1.08e-009; P < 0.0001). ISH demonstrated MRAP and MC2R expression in the adult rat zona fasciculata (ZF), (in cells capable of glucocorticoid synthesis), with highest concentrations in the undifferentiated zone (in cells incapable of glucocorticoid synthesis). MRAP2 was expressed at low levels throughout the adrenal cortex. Real-time qPCR of adrenal cDNA demonstrated a 20-fold greater expression of MRAP compared with MRAP2.

**Discussion**

- MRAP2 enables MC2R function only when stimulated with supraphysiological ACTH concentrations.
- MRAP expression is 20-fold greater than MRAP2 expression in the human adrenal gland.
- MRAP2 is expressed throughout the rat adrenal cortex. Its role in the adrenal gland remains unclear.
- Adrenal MRAP and MC2R expression is confined to the ZF and highest expression is seen in the undifferentiated zone of the cortex and suggesting ACTH may have a role in adrenal cell differentiation and migration.
P307
Epidemiology of Addison’s disease in the area of Banbury, Oxfordshire
John Komninos, Sybil Kohler, Niki Karavitaki & John Wass
Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, Oxfordshire, UK.

Introduction
Addison’s disease (AD) is a rare condition (reported prevalence of 40–110 per million) that requires prompt recognition and optimal management to prevent the risks associated with cortisol deficiency.

Aim
To assess the prevalence, presentation and clinical course of patients diagnosed with AD in the geographical area of Banbury in Oxfordshire.

Patients and methods
Notes of subjects with AD currently registered in 15 of the Surgeries in the area of Banbury were reviewed. All cases of AD were found following an exhaustive computer database search of agreed terms by the staff of each Practice.

Results
Amongst a population of 81,225 inhabitants, 15 patients (8 females/7 males) were diagnosed with AD, yielding a prevalence of 185 per million. Median age of diagnosis was 36 years (range 10–71). Median time to diagnosis since the first manifestations of the disease was 9 months (range 0.5–42) and the most frequent ones were tiredness (64.3%) and weight loss (64.3%). At diagnosis, the most frequent manifestations were postural dizziness (85.7%), nausea or vomiting (78.5%) and abdominal pain (57.1%). Adrenal antibodies were identified in 7 (46.7%) patients. Other autoimmunities/endocrinopathies (most commonly thyroid disease and gonadal failure), suggested autoimmune adrenal insufficiency in 10 patients (prevalence of 123 per million). In two cases cause was adrenal hemorrhage, one was diagnosed with haemochromatosis, while in two the cause remained unclear. Median follow up period was 10 years (range 1.8–46.3). Mean daily hydrocortisone and fludrocortisone replacement dose at last assessment was 22.3 mg and 96.2 ug, respectively. Most frequent complaints on follow up were excessive weight gain (33.3%) and tiredness (33.3%).

Conclusions
Prevalence of AD in Banbury is higher than described in literature and in line with recent reports suggesting rising of its incidence. Alertness on early manifestations can reduce diagnostic delay and risks associated with unrecognized cortisol deficiency.

P308
Derivatization of estrogens enhances sensitivity of analysis by liquid chromatography tandem mass spectrometry
Natalie Homer, Diego Cobice, Fraser Gibb, Gregorio Naredo, Scott Denham, Brian Walker & Ruth Andrew
University of Edinburgh, Edinburgh, UK.

Circularizing estrogens decrease after the menopause from 60–400 to 5–30 pg/ml in postmenopausal women and in men are below 30 pg/ml. These low physiological concentrations present an analytical challenge. Analysis of steroids by tandem mass spectrometry is attractive due to its high specificity compared with immunnoassays. However, estrogens do not ionise efficiently and therefore mass spectrometry is attractive due to its high specificity compared with immunnoassays. However, estrogens do not ionise efficiently and therefore derivatisation using FMP and dansyl chloride may be used for quantitative analysis of estrogens in men and postmenopausal women. The sensitivities achieved using these agents was comparable but enhanced selectivity was achieved using FMP since the product ion relates to the steroid moiety. This approach could be extended to include other phenolic steroids.

P309
Utility of basal DHEAS measurement in the detection of subclinical autonomous glucocorticoid hypersecretion in adrenal incidentaloma
Anand K Annamalai1,2, Narayanan Kandasamy1,2, Natalie Freeman1,2, Kuhan Venugopal1,2, Jonathan Chiu1,3, Ashley Shaw1,3, Helen L Simpson1,2, David Halsall1,4 & Mark Garnell1,2
1Addenbrookes Hospital, Cambridge, UK; 2Wolfson Diabetes and Endocrine Clinic, Cambridge, UK; 3Department of Radiology, Cambridge, UK; 4Department of Biochemistry, Cambridge, UK.

Background
Adrenal incidentalomas (AI) are identified in 4–7% of patients >40 years undergoing abdominal CT/MRI. Evidence of subclinical autonomous glucocorticoid hypersecretion (SAGH) is found in 5–10% of cases depending on the diagnostic criteria/thresholds adopted.

Aim
To examine the utility of basal DHEAS measurement in the detection of SAGH in a cohort of patients with AI.

Methods
Ninety-six consecutive subjects (49 females, 47 males: mean age 62 years, range 29–84 years) were referred to our clinic over a 3-year period. All patients were assessed by two clinicians (AA and MG), and underwent repeat dedicated adrenal imaging and biochemical profiling, including overnight dexamethasone suppression testing (normal cortisol suppression: <50 nmol/l). Cases in whom there was no clinical or biochemical evidence of endocrine hyperfunction, and with appropriate radiological features, were categorised as non-functioning adrenal adenomas (NFAA). SAGH was confirmed by the finding of low ACTH levels, abnormal circadian rhythm, and failure of cortisol to suppress during a 48 h low dose dexamethasone suppression test (normal: <50 nmol/l). DHEAS levels were classified as undetectable, suppressed (but detectable) and normal.

Results
Amongst NFAA (n=41), DHEAS levels were normal in 38, suppressed in one, and undetectable in two subjects. Amongst SAGH (n=18) subjects, nine had undetectable DHEAS, three had suppressed, and six normal levels. Receiver operating characteristic analysis using DHEAS concentration to diagnose SAGH returned an area under the curve of 0.85 (0.75–0.96). At the optimum cut-off of 0.9 nmol/l, sensitivity and specificity were 78% (52–94%) and 74% (58–87%) respectively. At a cut-point of 0.5 nmol/l the test is specific (95%) with a sensitivity for the detection of SAGH of 50%.

Conclusions
The finding of a subnormal DHEAS level in a patient with an AI is an important pointer to the potential presence of SAGH and merits further investigation.

P310
Effects of topical betamethasone and calcipotriol therapy in improving the tolerogenic potential of a ‘vaccine’ for type 1 diabetes
Mohammad Alhadj Ali1,2, Sally Thrower1, Stephanie Harris2, Susan Wong1 & Colin Dayan1
1Henry Wellcome Laboratories, University of Bristol, Bristol, UK; 2Department of Cellular and Molecular Medicine, University of Bristol, Bristol, UK.

Introduction
Peptide immunotherapy has been shown to be a simple and effective method of restoring tolerance and reversing disease in animal models of type 1 diabetes. It is highly important to administer the peptide interadermally into an environment in which antigen presenting cells such as dendritic cells are maintained in a tolerogenic state.

Objective
Our aim was to determine whether topical pre-treatment of the skin has the potential to enhance tolerogenic immunity during peptide immunotherapy.

Endocrine Abstracts (2011) Vol 25
P311

Hepatic 11β-hydroxysteroid dehydrogenase type I expression is dynamically related across the liver lobule and is linked to metabolic status
Adeeba Ahmed, Nina Semjonous, Elizabeth Rabbitt & Paul Stewart
University of Birmingham, Birmingham, UK.

Nearly all the functions of the liver display zonation in distribution within each the lobule. Hepatic cortisol availability is controlled by enzymes that generate cortisol from inactive cortisol (11β-hydroxysteroid dehydrogenase type 1, 11β-HSD1). Dysregulation of hepatic 11β-HSD1 activity has been implicated in insulin resistance. Key processes such as gluconeogenesis are located in the perportal hepatocytes, although current dogma describes hepatic 11β-HSD1 to show strong pericentral zonation.

11β-HSD1 immunohistochemistry and laser capture microdissection studies for mRNA expression on normal human liver samples showed strong pericentral staining and variable degrees of perportal staining in different samples. It was speculated that this may be related to hepatic energy state.

Male C57BL/6 mice age 10 weeks (6 per group) had livers harvested after timed fast of 0, 2, 4, 8 and 12 h, and refed for 12 h following a 12 h fast. 11β-HSD1 protein expression was increased throughout the liver parenchyma as well in pericentral areas, in addition to intense perportal staining of hepatic 11β-HSD1 in the fasted mice. Perportal expression was minimal in livers from fed and refed mice.

This zonation of 11β-HSD1 expression is entirely novel but may account for some of the known actions of GC in the liver. This may account for some of the known actions of GC in the liver. Perportal cortisol generation during fasting would promote metabolic processes including gluconeogenesis. Conversely, in the fed state, pericentral cortisol generation would promote pericentral metabolic functions including lipogenesis. This strongly implicates hepatic 11β-HSD1 in the regulation of key hepatic metabolic functions in health, with of course the possibility of dysregulation having impact upon metabolic disease states including insulin resistance and diabetes mellitus. At a molecular level, dynamic hepatic 11β-HSD1 expression has key implications upon the regulation of hepatic ER nutrient signalling. These concepts represent a significant paradigm shift in the thus far understood role and expression of hepatic 11β-HSD1.

P312

Prevalence of overt and subclinical hypothyroidism among Saudi pregnant women attending two referral hospitals in Saudi Arabia and associated maternal and fetal complications
Inass Taha & Jihan Alhazmi
Taibah University, Almadinah, Saudi Arabia.

Background
Thyroid disorders are among the common endocrine problems in pregnant women. Overt and subclinical hypothyroidism has been shown to be associated with an adverse outcome for both the mother and offspring.

Methods
Healthy volunteers received one of three pre-treatment regimes twice daily for 4 days to the skin of a small area of the arm: 0.05% betamethasone (S), 50 μg/mg calcipotriol (D), 0.05% betamethasone + calcipotriol (S+D). 12 h later, all subjects had a 10–15 mm suction blister raised at the site of treatment. Blister fluid was then withdrawn for the measurement of cytokine production. The blister roof was removed and epidermal cells isolated for study using 9-colour flow cytometry and for functional analysis in a mixed lymphocyte reaction (MLR) using allogenic PBMC.

Results
Epidermal cells comprised around 2% of the CD1a+ dendritic cells (EDC). In S subjects MLR proliferation was markedly suppressed with an associated reduction in interferon-γ (IFN-γ), interleukin 2 (IL2), and IL13 in the blister fluid. However, IL10 remained constant. A reduction in expression of CD86 by EDC. In S+D, 12 h later, all subjects had a 10–15 mm suction blister raised at the site of treatment. Blister fluid was then withdrawn for the measurement of cytokine production. The blister roof was removed and epidermal cells isolated for study using 9-colour flow cytometry and for functional analysis in a mixed lymphocyte reaction (MLR) using allogenic PBMC.

Conclusion
Pretreatment of the skin for as little as 4 days appears to have marked effects on proliferative responses but reduced expression of CD86 by EDC.

P313

Hashimoto’s encephalopathy: an unusual cause for coma
Aswathiah Srinath & Serife Mehmet
Queen Marys Hospital, Sidcup, Kent, UK.

A 58-year-old female with long standing type 1 diabetes and treated hypothyroidism presented to casualty with a 1-day history of vomiting and breathlessness. Clinically she was dehydrated with a depressed conscious level (GCS 12). Initial investigations confirmed diabetic ketoacidosis (pH 6.8, bicarbonate = 4.7 mmol/l, urinary ketones = +++, blood glucose = 34.5 mmol/l) and acute renal failure, likely to be secondary to pneumonia as there was consolidation on her CXR and a raised WCC. Despite correcting her acidosis and electrolyte disturbances and treating her sepsis she remained drowsy and was subsequently ventilated. Abnormal bodily movements were observed. Brain MRI was normal and EEG showed generalised slow waves with diffuse cortical dysfunction without any epileptiform features. Lumber puncture showed raised CSF protein with negative viral PCR studies. Abnormal admission TFTs were noted: FT4 = 8.0 pmol/l (NR: 9.0–19.0), TSH = 0.72 mU/l (NR: 0.35–4.94).

Thyroid microsomal antibodies were raised: 113 IU/ml (NR < 40). Four months prior to admission TFTs were normal (FT4 = 15.3 pmol/l, TSH = 0.48 mU/l) on 100 μg of thyroxine. TFTs may be in keeping with the sick euthyroid syndrome but hashimoto’s encephalopathy was considered especially as other causes for coma were excluded. Subsequently she was commenced on intravenous Methylprednisolone and gradually ventilatory support was weaned off. A full recovery was made. Hashimoto’s encephalopathy is a rare condition characterised by non-specific neurological and/or psychiatric symptoms often associated with high serum and/or CSF levels of anti-thyroid antibodies, increased CSF protein concentration, non-specific diffuse EEG abnormalities, and responsiveness to treatment with steroids. Many cases go undiagnosed.

Hashimoto’s encephalopathy should be considered in ‘investigation negative encephalopathies’ as it is responsive to steroid therapy and is readily reversible. Normal or slightly abnormal TFTs do not exclude the diagnosis. The association with type 1 diabetes is not well reported.

P314

Carbamazole embrocphy: implications for the choice of antithyroid drugs in pregnancy
Pamela Bowman, Nigel Osborne, Rachel Sturley & Bijay Vaidya
Royal Devon and Exeter Hospital, Exeter, UK.

Background
Maternal thyrotoxicosis affects 0.2% of pregnancies. Pharmacological treatments include carbamazole, methimazole and propylthiouracil. The Endocrine Society

Objective
Are to find out the prevalence and complications of overt and subclinical hypothyroidism among pregnant Saudi women living at ALMadinah region.

Materials and methods
A hospital-based prospective study performed at Madina Maternity and Children hospital and Ohud hospital, were Nine hundred and thirty six pregnant women between 12 and 30 weeks of gestation enrolled between July 2009 and June 2010. All women received the usual anti natal care. TSH level estimation was done and if TSH level was deranged then free T4, free T3 levels were added. Patients were managed accordingly.

Results
(9.3%) pregnant women were found to have overt hypothyroidism, and (14.9%) were diagnosed as subclinical hypothyroidism, reflecting high prevalence rate for both disorders. There was a high rate of caesarean section (35.6%) for women with overt hypothyroidism and of (30.2%) for women with subclinical hypothyroidism. Gestational DM developed in (23.5%) of women with overt hypothyroidism and (34.5%) with subclinical hypothyroidism. Intratravelle foetal deaths complicated (3.4%) of overt hypothyroid pregnant women, a low apgar score at delivery was encountered in (16.1%) of neonates of overt hypothyroid mothers and (10%) of neonates of subclinical hypothyroid mothers.

Conclusion
Pregnancy was associated with a high prevalence rate of subclinical and overt hypothyroidism among pregnant Saudi women. Significant adverse foetal and maternal outcomes may be prevented by screening all Saudi pregnant women as early as first antenatal visit by simple TSH testing.
recommends the use of propylthiouracil as first line during pregnancy, because of possible associations between carbimazole and congenital anomalies. However, recent reports link propylthiouracil to liver injury in adults, children, pregnant women and fetuses, raising questions over its safety.

Case
Our case is a 1-year-old girl who was exposed to carbimazole in utero due to treatment of maternal Graves’ disease. At conception her mother was taking 40 mg carbimazole with 100 µg thyroxine. On discovering she was pregnant, the thyroxine was stopped and the carbimazole gradually reduced to 10 mg for the remainder of the pregnancy. The mother remained euthyroid. The baby was born at 38 weeks gestation by forceps delivery. Birth weight was within the normal range at 2.7 kg. She was noted to have an atrial septal defect which was identified as a patent foramen ovale. This was surgically repaired at the age of 5 months. She also had a scalp defect consistent with aplasia cutis, characteristic facial features (large forehead, broad flat nasal bridge and thin upper lip) and persistent upper airway noise due to laryngomalacia. Further investigations excluded oesophageal atresia, tracheo-oesophageal fistula and chonal atresia.

Evidence
A review of English publications in Medline revealed 31 other cases of 2 or more congenital anomalies reported in babies born to mothers treated with carbimazole or methimazole in pregnancy. Anomalies in these cases include distinctive facial features (68%), oesophageal atresia (65%), aplasia cutis (29%), nipple deformities (23%) developmental delay (16%), patent venous tunnel (16%), tracheo-oesophageal fistula (13%), oesophageal atresia (13%) and omphalocele (6%).

Conclusion
Constellation of several congenital anomalies in our case and others supports the concept of a carbimazole embryopathy and the Endocrine Society advice to avoid carbimazole during the first trimester.

P316
The mystery of persistent thyrotoxicosis

Auditi Naziat1, Mohgah Elsheikh1,2 & Gearoid Kingston2
1Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK; 2Centre for Endocrinology, Royal Berkshire Hospital, Reading, UK.

We report an unusual case of struma ovarii diagnosed after thyroidectomy for Graves’ disease and papillary thyroid cancer.

A 46-year-old woman presented with a 3-month history of weight loss, palpitations and irregular periods. Physical examination revealed tachycardia, fine goitre and a diffuse thyroid uptake >0.1 (0.5–4.2 mU/l), FreeT4 26 (10.8–19.3 pmol/l) confirmed thyrotoxicosis.

Thyroid ultrasound scan showed a diffusely enlarged gland with an 8 mm solitary nodule in the left lobe and scintigraphy of the neck revealed diffusely increased uptake. Fine needle aspiration cytology of the thyroid nodule was suspicious of a follicular lesion (THY 3). She underwent a total thyroidectomy. Histology revealed a papillary thyroid carcinoma, stage pT4 N1 Mx and the rest of the gland was consistent with Grave’s disease. Shortly after receiving Radio iodine ablation for the thyroid cancer she became thyrotoxic (TSH <0.01, FT4 54, FT3 13). Post ablation scan showed increased uptake in the lower abdomen. A CT scan of the pelvis revealed a 13 cm mass originating from the right ovary. She underwent a right salphingo- oophorectomy. The histology of the ovary showed struma ovarii consistent of focally hyperplastic benign thyroid tissue which had completely replaced the normal ovary.

She remained euthyroid on levothyroxine replacement. Reports of coexisting Graves’ disease and struma ovarii or papillary carcinoma and struma ovarii are very rare and we believe this is the first case report confirming the clinical and pathological features of benign struma ovarii. Graves’ disease and papillary thyroid carcinoma in the same patient. Only 5–15% of women with struma ovarii develop hyperthyroidism. In our patient it is likely that the high iodine load given for radioablation triggered thyroid tissue hyperactivity in the struma ovarii and subsequent thyrotoxicosis. Ovarian metastasis from thyroid cancer is very rare and in our case there was no such histology evidence.

P315
Outcomes of radioactive iodine treatment for hyperthyroidism: 1 year follow up survey in subjects attending a general hospital endocrine clinic

Rajesh Rajendran, Ramona Verdaguer & David Coppini
Poole General Hospital, Poole, UK.

Aim
Retrospective survey on outcomes of 131I therapy in subjects with hyperthyroidism.

Methods
We analysed the outcomes at 1 year of 55 episodes of 131I therapy in 52 patients (36 males, 15 females) who were treated between January 2007 and January 2009.

Results
At 1 year post 131I therapy, 31 (56.3%) subjects were hypothyroid (median age 54 years), 14 (25.5%) were euthyroid (median age 69 years) and 10 (18.2%) remained hyperthyroid (median age 73 years). 31 subjects (56.3%) had Graves’ disease, 20 (36.4%) had multinodular goitre (MNG) and 4 (7.3%) were not classified.

In 39 female subjects treated with 131I, 22 (56.4%) became hypothyroid, 10 (25.7%) remained euthyroid and 7 (17.9%) became hyperthyroid. Amongst the 16 male subjects, 9 (56.2%) became hypothyroid, 4 (25%) remained euthyroid, and 3 (18.8%) became hyperthyroid.

Out of 31 subjects who became hypothyroid, 21 (67.8%) had received 350 MBq, 9 (29%) had received 550 MBq and 1 (3.2%) had received 650 MBq. Out of 14 subjects who were rendered euthyroid, 5 (35.7%) had 350 MBq, 8 (57.1%) had 550 MBq and 1 (7.1%) had 620 MBq. In the 10 subjects who remained hyperthyroid after 131I therapy, 5 (50%) had received 350 MBq and 5 (50%) had 550 MBq.

Thirteen subjects became profoundly hyperthyroid (TSH >50) within one year. Of these, 10 (77%) had Graves’, 2 had MNG (15%) and 1 (8%) was not classified. 7 subjects (54%) had received 350 MBq whereas 6 (46%) had received 550 MBq of 131I therapy.

Conclusion
Despite the relatively small sample size, age, sex, aetiology and dose of 131I did not seem to influence the outcome of 131I therapy. Although larger studies are needed to confirm our observations, our series suggests an increased risk of profound hyperthyroidism following 131I in subjects with Graves’ disease when compared to subjects with MNG.

P317
Anaplastic thyroid carcinoma presenting with stridor after ablative radioliodine therapy for Graves’ disease

Sajid Kalathil, Atif Munir & Sath Nag
James Cook University Hospital, Middlesbrough, UK.

Anaplastic thyroid carcinoma accounts for <5% of thyroid cancer. The simultaneous occurrence of anaplastic carcinoma (ATC) and Graves’ thyrotoxicosis is extremely uncommon with only six cases described in the literature. A 73-year-old female presented with weight loss and atrial fibrillation. Investigations showed TSH 0.02 mU/l with T4-40 pmol/l and T3-16 pmol/l suggesting thyrotoxicosis. There was no thyromegaly or palpable nodule at presentation. TSH was elevated at 10.3 U/l suggesting Graves’ disease. Carbimazole was commenced with good biochemical response followed by a 400 MBq dose of ablative 131I. Post radionuclide thyrotoxicosis was managed with levothyroxine. She presented four months later with severe respiratory distress. An anterior indurated neck mass was noted. CT showed a diffusely enlarged, heterogeneous thyroid gland with calcification causing laryngeal stenosis. Bronchoscopy revealed tracheal stenosis below the vocal cords due to external compression and tumour infiltration. FNAC was initially suspicious for papillary thyroid carcinoma but subsequent core biopsy immunohistochemistry was consistent with anaplastic thyroid carcinoma. Surgical debulking of the tumour was planned and an elective tracheostomy performed. However her condition deteriorated acutely due to a respiratory tract infection and concurrent acute coronary syndrome. Palliative care was instituted and the patient died a week later.

Discussion
Our case initially presented with Graves’ disease with no thyromegaly or palpable nodules and developed a massive tumour with compressive symptoms 8 months later. 12 to 26% of patients with GD are reported to have palpable thyroid nodule and 33.6% when detected ultrasonographically. Malignancy rate of nodule is 10–17% and becomes higher if the nodule is palpable or scintigraphically cold. Most carcinomas associated with Graves’ disease are papillary. There are case reports which show association with struma ovarii and papillary thyroid carcinoma in the same patient. Only 5–15% of women with struma ovarii develop hyperthyroidism. In our patient it is likely that the high iodine load given for radioablation triggered thyroid tissue hyperactivity in the struma ovarii and subsequent thyrotoxicosis. Ovarian metastasis from thyroid cancer is very rare and in our case there was no such histology evidence.
P318

Serum phosphate predicts temporary hypocalcaemia following thyroidectomy
Amir H Sam, Waljd S Dhill, Mandy Donaldson, Ahmad Moolla, Karim Meeran, Neil S Tolley & Fausto Palazzo
Imperial College London, London, UK.

Background
Temporary hypocalcaemia occurs in up to 40% of patients following a total thyroidectomy. Serum calcium and parathyroid hormone (PTH) measurements are currently used to predict post-thyroidectomy hypocalcaemia. However, immediate access to PTH measurement is expensive and not widely available. Serum phosphate responds rapidly to changes in circulating PTH levels and its measurement is readily available in all hospitals. We evaluated the use of serum phosphate to predict temporary hypocalcaemia post-thyroidectomy.

Methods
We retrospectively assessed 111 consecutive patients who had total or completion thyroidectomy. Patients had serum calcium and phosphate measured pre-operatively, on the evening of surgery (day 0), on the morning of day 1 and over the following week as clinically indicated. Serum PTH was measured on the morning of day 1. Vitamin D levels were measured pre-operatively.

Results
Seventy-six patients did not develop treatment-demanding hypocalcaemia. In these patients, the mean serum phosphate on the morning of day 1 was lower than that of on the evening of surgery. Seventeen patients with a vitamin D level > 25 nmol/l developed hypocalcaemia requiring treatment from day 1 onwards. All had an overnight rise in serum phosphate to > 1.44 mmol/l (100% sensitivity and specificity for predicting hypocalcaemia). Twelve patients who had a vitamin D < 25 nmol/l also developed hypocalcaemia but had an attenuated rise in serum phosphate.

Conclusion
Serum phosphate is a reliable biochemical predictor of post-thyroidectomy hypocalcaemia in patients without vitamin D deficiency. Use of serum phosphate may facilitate safe day 1 discharge of patients undergoing thyroidectomy.

P319

Audit on follow up of thyroid function tests done during osteoporosis screening
A Santhakumar, H Sugathan & D Woods
Wansbeck General Hospital, Northumberland, UK.

Background
National osteoporosis guideline recommend routine measurement of thyroid function tests. However it is well recognised that abnormal thyroid function tests done during acute hospital stays is not always suggestive of thyroid dysfunction and that they need to be repeated to determine the need for further management.

Aim
To identify and assess the follow up of thyroid function tests (TFTs) done between June 2009 and Feb 2010 in the orthopaedic ward of our hospital as part of osteoporosis screening.

Method
TFTs requested from the orthopaedic ward at our hospital between June 2009 and Feb 2010 were identified via the pathology lab database. Abnormal TFTs were followed up on the hospital database to determine if they had been repeated, investigated further or referred to the endocrine service.

Results
Three hundred and three patients (65 males, 258 females) had TFTs requested from the orthopaedic ward during the specified period. 59/303 patients had initially abnormal TFTs. 29 patients had isolated suppressed TSH mean 0.28 (range 0.04–0.38) and 26 patients had isolated raised TSH mean 8.4 (range 5.2–37). 4 patients had suppressed TSH with elevated free thyroid hormones (free T4 range 13.9–32). 205/95 patients had repeat TFTs while 39 patients had no recorded repeat tests on the hospital database. The median interval of repeat TFTs was 6 weeks (range 3 days to 12 weeks). None of the patients with abnormal TFTs had thyroid antibodies done and only one was referred to endocrine services.

Conclusion
There is a wide variability in the follow up of thyroid function tests done for osteoporosis screening with majority of patients not having follow up investigations. There needs to be more awareness among the health professionals about the appropriate follow up of thyroid function tests done in acute hospital setting.

P320

Serum homocysteine concentrations in hypothyroid, euthyroid and hyperthyroid patients
Dariusz Kajdaniuk1, Bogdan Marek1, Danuta Niedziolka1, Elzbieta Swietochowska1, Gani Zeka1, Mariusz Nowak1, Lucyna Sieminska1, Joanna Glogowska-Szlez1, Beata Kos-Kudla1 & Zofia Ostrowska1,2
1Department of Pathophysiology and Endocrinology, Medical University of Szczesia, Zarbze, Poland; 2Department of Clinical Biochemistry, Medical University of Szczesia, Zarbze, Poland.

Background
Thyroid hormone deficiency is associated with increased cardiovascular morbidity, which cannot be fully explained by the atherogenic changes in lipid profile observed in these patients. Hypothyroïdism can help to explain the increased risk for arteriosclerotic coronary artery disease in hypothyroidism because is an important and independent risk factor for cardiovascular disease. Hypothyroidism decreases hepatic levels of enzymes involved in the remethylation pathway of homocysteine.

Aim
Hypothyroid (TSH > 10 mIU/L, low T4, and T3) were compared with hyperthyroid patients and euthyroid control to examine the relationship among homocysteine serum levels and thyroid hormonal status.

Material
Of 99 patients with hypothyroidism (27–59 years of age): 44 with autoimmune thyroiditis and 54 after thyroidectomy for thyroid multinodular goiter or carcinoma. 63 patients with hypothyroidism (23–61 years of age): 28 with toxic nodular goiter and 35 with Graves’ disease. Control group was consisted of 30 healthy volunteers.

Methods
Homocysteine serum concentration was measured by fluorimetric HPLC.

Results
Serum homocysteine levels were significantly higher in patients with hypothyroidism in comparison with those of healthy controls (19.1 ± 8.4 vs 10.2 ± 2.7 mmol/l, P < 0.05). Homocysteine levels were positively related to TSH blood concentrations and inversely related to folicates. Thyroid hormone replacement significantly decreased homocysteine serum levels. There were no differences in homocysteine levels between patients with hyperthyroidism and healthy controls.

Conclusion
We found an increase in serum homocysteine during hypothyroidism. The increase in serum homocysteine concentration may confer increased cardiovascular risk in hypothyroid patients.

P321

PITTG-binding factor (PBF) induces hyperplastic lesions in thyroids of aged mice
Martin Read1, Greg Lewy1, Neil Sharma1, Jim Fong1, Vicki Smith1, Gavin Ryan1, Robert Seed1, Perkin Kwan1, Wendy Leadbeater1, Adrian Warfield1, Jeffrey Knud1, John Watkinson1, Jayne Franklyn1, Kristien Boelaert1 & Chris McCabe1
1University of Birmingham, Birmingham, West Midlands, UK; 2University Hospitals Birmingham NHS Foundation Trust, Birmingham, West Midlands, UK; 3Memorial Sloan-Kettering Cancer Center, New York, New York, USA.

Previously we showed that thyroid-targeted overexpression of the PITTG binding factor (PBF) induced significant goitrogenesis in transgenic mice (PBF-Tg). In 10 week old PBF-Tg mice thyroid weight was increased by 1.8 ± 0.3-fold compared to wild-type (WT) controls (n = 20, P < 0.001). We have now examined the long-term effects of PBF overexpression on thyroid gland weight and histology in aged PBF-Tg mice. Our study shows that the penetrance of increased thyroid weight was 100% with all PBF-Tg mice demonstrating markedly enlarged thyroid glands (n = 256). By 12 months of age PBF-Tg thyroid glands were 3.2-fold larger than that of WT littermates (n = 22, P < 0.0001), which persisted to 18 months of age (2.7 ± 0.5-fold, n = 27, P < 0.0001). Histological examination showed that PBF-Tg mice were prone to macrofolicular lesions with > 90% of mice (n = 11/12) demonstrating follicles > 250 microns in diameter by 18 months of age (P = 0.02 compared with WT). Focal and nodular hyperplasia was also apparent, with 75% of PBF-Tg mice (n = 9/12) demonstrating evidence of hyperplastic lesions by 18 months of age (P = 0.009 compared with WT). Western blot analysis showed a significant 3.4 ± 1.2-fold (n = 7, P = 0.0007) activation of phosphorylated Akt, a known regulator of thyroid cell proliferation, in transgenic thyroids. In contrast there was no significant increase in total Akt levels. Positive
immunostaining with the proliferation marker cyclin D1 in diffuse goitre regions was significantly higher in aged PBF-Tg mice (n=4834/35958 positive cells) compared to WT mice (n=1231/25987 positive cells; P<0.0001). Further, hyperplastic lesions of aged PBF-Tg mice demonstrated much greater cyclin D1 staining, ranging from 19 to 68% of cells, with a mean value of 37.5±20.7% (n=5). These results demonstrate that targeted overexpression of PBF induces goitre in vivo, and causes significant hyperplastic growth of the thyroid gland. Further, our findings implicate the Akt pathway in mediating PBF-induced thyroid cell proliferation in vivo.

The remaining two patients developed transient hyperthyroidism, later reverting to permanent hypothyroidism requiring levothyroxine. We detected clinically relevant thyroid dysfunction at a frequency similar to previous studies. Thyroid dysfunction was more common in female patients and those of Eastern European origin. All patients continued IFNα treatment and euthyroid status was achieved with medical intervention. These data suggest that frequency of TFT testing could possibly be reduced in male patients and may need to be increased in Eastern European patients. Those who develop thyroid dysfunction can be managed without discontinuation of IFNα therapy.

P324
Effect of weight loss through gastric bypass on thyroid function in euthyroid people with morbid obesity
Antonia MacCuish¹, Salman Razvi² ³ & Akheel Syed¹ ⁴
¹University of Manchester, Manchester, UK; ²Newcastle University, Newcastle upon Tyne, UK; ³Queen Elizabeth Hospital, Gateshead, UK; ⁴Salford Royal Hospital, Salford, UK.

Background
Thyroid function within the normal range has been shown to influence body weight in a population. Even slightly elevated serum TSH levels are associated with an increase in the occurrence of obesity. It is unclear whether significant weight loss has the reverse effect on thyroid function, and studies to date have yielded inconsistent results.

Aims
Our aim was to describe changes in TSH and T4 in relation to durable and significant weight loss in obese people with normal thyroid function before and after Roux-en-Y gastric bypass (RYGB) surgery.

Methods
We conducted a retrospective cohort study in 55 patients (13 men) who had undergone RYGB. Data recorded included TSH, FT4 and excess weight loss at baseline and median 4.5, 15 and 24 months after RYGB. Means were compared by t-test.

Results
Patients had a mean (s.e.m.) preoperative BMI of 48.1 (1.58) and achieved significant weight loss over time. Mean (s.e.m.) baseline serum TSH levels was 2.00 (0.14) mIU/l with no significant change over time (2.02 mIU/l at 24 months). Mean (s.e.m.) baseline FT4 concentrations were 13.02 (0.42) pmol/l and increased significantly following bariatric surgery to 15.20 (0.57) pmol/l at 24 months (P<0.0001).

Conclusion
Weight loss after RYGB is accompanied by significant increase in FT4 but no change in TSH. Further work is required to address the mechanism of alteration in the pituitary-thyroid axis after weight loss.

P325
Iodine induced thyrotoxicosis: the danger of over the counter slimming aids
Ravikumar Bachuwar & Mark Freeman
Dewsbury District Hospital, Dewsbury, UK.

Over recent years, the numbers of commercially available slimming aids have increased dramatically. Whilst the majority of these aids are harmless, their interaction with normal physiology is either not understood or not brought to the attention of the customer. We report the case of a 45-year-old woman who presented with clinical and biochemical thyrotoxicosis (fT4 31.5 pmol/l, fT3 14.3 pmol/l, TSH <0.02 mIU/l). She had elevated TPO antibodies (51 IU/ml). She denied taking any prescription or homeopathic medication or iodine containing compounds. There was no history of intravenous contrast use. Her thyroid function settled rapidly suggesting a thyroiditis rather than Graves disease as the underlying cause for her condition. At her follow up appointment, she asked if she could continue to take an over the counter slimming aid (Tesco slimming aid 60s, one tablet taken three times per day), having started this therapy 4 months previously. She was advised against this. According to the product literature, this slimming aid contains bladderwrack (Fucus vesiculosus), a commonly used food supplement, is a significant source of iodine. This is known to increase the risk of thyrotoxicosis via the Jod-Basedow phenomenon.

The accompanying product literature advises against using these tablets in the presence of thyroid disease. However, the majority of patients will be unaware of their thyroid status. This case reinforces the need to take a full dietary history in

Endocrine Abstracts (2011) Vol 25
patients with thyrotoxicosis. Furthermore, patients wishing to take over the counter medication should be advised to seek medical advice before doing so.

### P326

**The effect of parathyroidectomy on neuropsychological symptoms in patients with asymptomatic primary hyperparathyroidism**

Hassan Kahal1, Mo Aye1, Alan Rigby1, Thozhukat Sathyapalan1, James England2 & Stephen Akin1

1Academic Endocrinology, Diabetes and Metabolism, Hull York Medical School, Hull, UK; 2Department of Diabetes, Endocrinology, and Metabolism, Hull Royal Infirmary, Hull, UK; 3University of Hull, Hull, UK; 4Department of Otolaryngology Head and Neck Surgery, Hull Royal Infirmary, Hull, UK.

**Introduction**

With increased biochemical screening primary hyperparathyroidism (pHPT) is discovered incidentally while patients are still asymptomatic.

**Objective**

To assess the impact of surgery on neuropsychological symptoms in people with apparent asymptomatic pHPT.

**Methods**

Twenty-four patients with asymptomatic pHPT requiring parathyroidectomy, in accordance with NIH recommendations, were recruited prospectively between May 2005 and August 2007. The control group of 23 subjects was recruited simultaneously from consecutive patients undergoing diagnostic hemiroidectomy (HT) for cold thyroid nodules. Operations were performed by a single surgeon.

Biochemical investigations and quality of life (QoL), using the hospital anxiety and depression scales, were measured preoperatively and 3 months post-operatively. Data are presented as mean and s.d. and analysed using Student’s t-test.

**Results**

At baseline, patients with pHPT compared to the HT group were older, average age (± s.d.) 60.0 (± 13.36) vs 43.0 (± 14.56) years, respectively (P < 0.001), had more females 93.1% (n = 27) vs 70.4% (n = 19), and higher scores only on the HAD-D (depression) scale, 14.1 (± 4.14) vs 11.0 (± 3.49), P = 0.0065.

Postoperatively, there was a significant improvement in QoL with a pre and post-operative mean change 1.0 (± 2.57), P = 0.001 on HAD – A, 2.79 (± 3.85), P = 0.0017 on HAD – D, and 3.2 (± 4.57), P = 0.0022 on MRS in patients with pHPT. In the HT group; HAD – A mean change 1.0 (± 3.8), P = 0.22, HAD – D – 0.173 (± 3.60), P = 0.786; and MRS – 0.086 (± 4.1), P = 0.919.

Postoperatively, calcium andPTH normalised in all patients in the pHPT group; 2.44 (± 0.09) mmol/l and 49 (± 2.17) ng/l, respectively. Calcium levels remained normal in the HT group. There were no changes in CRP, uric acid, and malondialdehyde levels in either group.

**Conclusions**

Apparent asymptomatic pHPT is associated with neuropsychological symptoms and a reduced quality of life that improve after parathyroidectomy.

### P327

**Management of hyperfunctioning thyroid malignancy with psychiatric co-morbidity**

David Till, Jackie Gilbert, Dylan Lewis, James Crane, Simon Aylwin & Alan McGregor

Kings College Hospital, London, London, UK.

A 70-year-old female with known schizophrenia presented in hyperthyroid crisis. Examination revealed muscle wasting, tremor, sweating, low-grade fever, and sinus tachycardia. Biochemistry confirmed the diagnosis (TSH <0.1 mIU/l, thyroxine 41 pmol/l (9.25), tri-iodothyronine 25 pmol/l (3.5–6.5)). The patient was commenced on i.v. esomolol and carbimazole (40 mg) crushed into warm milk.

However, lacking mental capacity, and refusing to take all medication, she underwent emergency thyroidectomy. The thyroid was recognised to be malignant intra-operatively, so total thyroidectomy and extensive lymph node dissection was undertaken. A pathological diagnosis of follicular thyroid carcinoma was made (FTC pT4c N0 (0/8) Mx STAGE IV).

Postoperative thyroid hormone levels proved difficult to control with high dose thionamides. CT and NM imaging revealed a large (8 × 10 × 12 cm), highly vascular, hepatic metastasis, as well as persistent neck disease. Radioiodine therapy was determined to be inappropriate, due to her overactive status, the likelihood of all 131I being retained in the hepatic lesion, and as the patient required one-to-one nursing due to her psychiatric condition.

The patient was unfit for hepatic surgery, so underwent percutaneous arterial embolisation, with the aim of reducing tumour blood flow and amelioration of symptoms. This has allowed palliative control of the thyrotropic state with carbimazole and thyroxine, and concomitantly improved the patient’s psychiatric condition. Her psychiatric team, and family (predominantly living abroad), were included in all stages of treatment.

This case presents an unusual history of thyroid follicular malignancy, coupled with significant psychiatric disease. Management decisions highlight the benefit of discussion though Network Multidisciplinary teams, and access to consideration of all available treatment options. Hepatic metastases are a relatively unusual feature of follicular thyroid cancer, but like other neuroendocrine hepatic disease may be hyperfunctioning. Cases have previously reported radiofrequency ablation or hepatic surgery as alternative approaches to their treatment.

### P328

**Thyrotoxic crisis: a stormy period on intensive care**

Dilip Eappen & Ryan D’Costa

Pinderfields General Hospital, Wakefield, Yorkshire, UK.

Thyroid storm or thyrotoxic crisis is a manifestation of an extreme state of thyrotoxicosis. It is both rare and potentially fatal. Its presentation can be clouded by the precipitating illness and the involvement of one or more organ systems underlining the importance of clinical suspicion, early recognition and prompt intervention.

We present a case of a 37-year-old man presenting to hospital with a community acquired chest infection that had not settled with oral antibiotics from his general practitioner. Twenty-four hours into the admission he suddenly deteriorated developing hypotension (BP 80/60 mmHg), fast atrial fibrillation and pulmonary oedema.

Past history included Graves’ disease but he had been non compliant with treatment for the preceding 8 months. Although he was initially thought to have sepsis secondary to pneumonia associated with cardiogenic shock, his thyroid function tests indicated severe thyrotoxicosis: TSH <0.05 (normal 0.2–4 mIU/l), free T3 29.9 (normal 2.5–5.7 pmol/l), free T4 46 (normal 9–24 pmol/l). This along with the clinical picture of thyrotoxic state was consistent with a diagnosis of thyroid storm. Prompt anti thyroid medications and supportive management on intensive care was instituted. This included carbimazole (later changed to propylthiouracil), Lugol’s iodine and high dose dexamethasone along with broad spectrum antibiotics, inotropic and ventilatory support. Recovery was complicated by liver dysfunction and renal failure requiring haemo-filtration for a short period.

His thyroid function improved alongside his response to treatment. Following 2 weeks on intensive care he made a remarkable recovery. After discharge his thyrotoxicosis was successively treated as an out-patient with two separate courses of radioiodine.

This case serves to remind us of this rare form of severe thyrotoxicosis. In this case it had occurred complicating sepsis. It also demonstrates the multiple organ systems that can be involved and the importance of prompt and aggressive multifaceted therapeutic intervention.

### P329

**Hypothyroidism and adrenoleukodystrophy, a rare association**

Venkata Katreddy, Ashraf Bdri, Azizul Azziz Al-Akkar & Khaled Ashawesh

Russells Hall Hospital, Dudley, Westmidlands, UK.

**Introduction**

Adrenoleukodystrophy is an X-linked disorder associated with functional defect of very long fatty acid (VLFCA) oxidation leading to accumulation of VLFCA in the white matter of brain and adrenal cortex. It usually presents with adrenal insufficiency and neurological problems and has association with other autoimmune conditions reported so far including vitiligo, ulcerative colitis. We report a case of adrenoleukodystrophy, vitiligo and hypothyroidism.

**Case**

A 60-year-old male was diagnosed with idiopathic epilepsy at the age 15 was referred to neurology clinic with right foot drop at the age of 20. MRI scan showed marked high signal changes in white matter in occipito-parietal regions bilaterally extending temporal regions, internal capsule and cerebellar peduncles consistent with degenerative disease. After long work up his VLFCA were found...
to be abnormally elevated. Genetic screening showed Deletion 2252-15del14, affecting the intron 8 splice receptor on X chromosome. Family screening was done. He was referred to our Endocrine clinic with symptoms of hypothyroidism and confirmed TSH12.4 mIU/l and borderline T4 of 10.3 pmol/l which was confirmed on repeat. Examination revealed small palpable goitre and marked vitiligo. His microsomal antibodies were just positive. He was started on thyroxin and continued on it. Later he was diagnosed with adrenal insufficiency and is currently on hydrocortisone and fludrocortisone.

Conclusion
Hypothyroidism in association with adrenoleukodystrophy and vitiligo is not reported so far. There is one case report in literature with adrenal myeloneuropathy with hypothyroidism but not vitiligo. It is difficult to establish the cause of hypothyroidism in this case whether it is due to adrenoleukodystrophy perse or due to autoimmune process as his microsomal antibodies were slightly positive. This case highlights the importance of looking for other autoimmune diseases in adrenoleukodystrophy as they have been reported increasingly.

P330
Should individuals with a Thy 3 result always undergo surgery?
Jessica Green, Toby Hunt, Rasha Mukhtar, Alexandra Ward, Kate Allen, Anthony Robinson & Paul Maddox
Royal United Hospital, Bath, UK.

Introduction
British Thyroid Association Guidelines state that the diagnosis of thyroid malignancy cannot be based on the results of an aspiration’s cytology alone and will usually require surgical removal for confirmation. There has been much debate about the management of indeterminate fine needle aspirations (Thy 3).

Recent publications have reported that the prevalence of thyroid carcinoma following a Thy 3 result to be as high as 28%, emphasising the importance of surgical management in such individuals. To assess the outcomes within our own practice, we performed a retrospective review of all patients undergoing thyroid surgery between January 2006 and April 2010 identifying those with initial histology of Thy3.

Results
A total of 235 thyroid surgeries were performed, of which 44 (19%), were identified as Thy 3 on cytology. Patient’s average age was 49 years with the majority being female 37 (84%). 15 (35%) patients underwent more than one aspiration. The average size of the nodules was 34 mm. Management decisions were made by a dedicated multidisciplinary team for those with cytological features of a follicular neoplasm (Thy 3).

A total of 30 (68%) individuals had benign lesions of which 9 were colloid, 15 follicular lesions and 6 dominant nodules on a background of multi-nodular goitre.

The remaining 14 (32%) were confirmed to have malignant histology. Unlike a recent series only 2 were follicular (14%), while the other 12 were papillary (86%). 3 (25%) were micro-papillary, which if excluded as being incidental, reduces the prevalence of carcinoma to 11 (25%).

Conclusions
The incidence of malignancy among our patients with Thy 3 cytology on fine needle aspiration is similar to reports by other institutions. Differences residing in the unexpected prevalence of papillary carcinoma within our series. The results add further weight to the recommendation that following a confirmed Thy3 cytology result surgical intervention should be advocated.

P331
The natural history of endogenous subclinical hyperthyroidism
Thenmalar Vadiveloo, Peter Donnan, Lynda Cochrane & Graham Leese
University of Dundee, Dundee, UK.

Objective
To define the rates of progression to frank hyperthyroidism and to normal thyroid function for subclinical hyperthyroid patients (SH).

Design
Record-linkage technology was used retrospectively to identify patients with SH in the general population from 1st January 1993 to 31st December 2009. Patients were defined as being hypertensive or having previously being prescribed thyroid hormone replacement therapy.

Patients
Patients were identified on the central computer records. The following variables were examined: TSH, T3, T4, and TSH/T4. The reference range for TSH was 0.4–4.0 mIU/l and for T4, 50–110 nmol/l. The patients were divided into 3 groups: grade 1: TSH between 0.4–4.0 mIU/l; grade 2: TSH between 4.0–10 mIU/l; and grade 3: TSH >10 mIU/l. All patients were followed up for a minimum of 6 months.

Results
A total of 2024 patients with SH were identified, a prevalence of 0.63% and an incidence of 29 per 100 000 in 2008. Most SH cases without thyroid treatment remained as SH at 2 (81.8%), 5 (67.5%) and 7 (63.0%) after diagnosis. Few patients (0.5–0.7%) developed hyperthyroidism at 2, 5 and 7 years. The percentage of SH cases reverting to normal increased with time: 17.2% (2 years), 31.5% (5 years) and 35.6% (7 years) and this was more common in SH patients with baseline TSH between 0.1 and 0.4 mIU/l.

Conclusion
Very few SH patients develop frank hyperthyroidism whilst a much larger proportion revert to normal, and many remain with SH.

P332
A case of amiodarone induced thyrotoxicosis, type II followed by subsequent hypothyroidism
Fred McElwaine & Ahmed Helmy
Erne Hospital, Enniskillen, UK.

Introduction
Amiodarone is a class III antiarrhythmic agent widely used in the treatment of atrial and ventricular arrhythmias. Amiodarone is known to cause thyroid dysfunction in three ways: amiodarone induced hypothyroidism, amiodarone induced thyrotoxicosis (AIT) type I and type II.

We present a case of a patient with AIT type II who subsequently developed hypothyroidism.

Case presentation
A 43-year-old man was admitted in December 2009 with increasing sense of hotness. At the time of his presentation TFTs were measured revealing a FT4 of 121 pmol/l, FT3 of 21 pmol/l respectively with TSH completely suppressed. He had a history of pulmonary and cardiac sarcoidosis, as a result of which he had episodes of wide complex tachycardia in 2007 for which he was commenced on Amiodarone with normal baseline and follow up thyroid function (TFTs). Examination revealed no tremors but he had a small, palpable diffuse goitre with no retrosternal extension and no thyroid eye disease.

Antithyroid antibodies were normal, as was a thyroid ultrasound scan. Subsequently he underwent a thyroid uptake scan which showed no significant accumulation of radioactive iodine within the thyroid. A diagnosis of Amiodarone induced thyrotoxicosis, type II was made. Amiodarone was replaced by bisoprolol and he was commenced on 40 mg prednisolone tapered gradually over 3 months. He became clinically and biochemically euthyroid until June 2010 when his TFTs revealed overt hypothyroidism: FT4 7.9 pmol/l, TSH 31.2 mU/l requiring thyroxine replacement therapy.

Discussion
While hypothyroidism and thyrotoxicosis in patients taking Amiodarone are seen relatively frequently it is uncommon for a patient to develop hypothyroidism after having previously been treated for AIT. This case emphasises the importance of following up patients with AIT even when they have become euthyroid and are off treatment.

P333
Audit of thyroid function testing in patients on amiodarone
Vidya Srinivas, Ramalingam Srinivasan & Joanne Randall
Jammes Paget University Hospital, Gorleston, Lowestoft, UK.

Objective
Amiodarone is an iodine rich, potent antiarrhythmic drug that is highly lipid soluble and total body iodine stores remain increased for up to 9 months. Abnormal thyroid functions, either thyrotoxicosis or hypothyroidism occur in up to 14–18% of patients receiving long-term amiodarone therapy. Hence regular thyroid function tests are required in patients on long-term amiodarone treatment. The BNF clearly states that thyroid function tests should be done at a minimum 6 monthly intervals.

Methods
We did a retrospective notes review on 75 consecutive patients prescribed amiodarone from April 2008, and followed up the thyroid function tests done on these patients, as well as the action taken on these findings.
Results
Of a total of 75 patients, 39 were men (52%). 39 patients were over 80 years. 12 patients had previous thyroid disease, (2 hyperthyroid treated, 1 amio related and 9 hypothyroid). 52 were new prescription started as inpatients of whom 26 had baseline TFTs, and 23 were follow ups of whom 11 had TFTs done within 6 months.
Of 44 patients had no appropriate follow up testing, 31 patients had testing done appropriately and 2 patients moved out of the area.
Interestingly, 2 patients had pulmonary complications and Amiodarone had to be withdrawn and 9 patients died within 6 months of whom, 2 had TFTs within 6 months. Of all TFT reports, 13 were abnormal, with 2 patients being hyperthyroid, 2 patients having no significant hypothyroid and 9 had a raised TSH with normal T4. Of these 13 patients, only 6 had appropriate TFTs.

Conclusion
Thyroid function testing at regular interval is only done in about 40% of prescriptions issued. Almost 17% of those who have regular thyroid testing did show abnormal values. Hence it is strongly advised that regular thyroid testing is encouraged in the outpatient as well as inpatient settings.

P334
Depot specific differences in regulation of hyaluronan production of relevance to Graves' orbitopathy
Lei Zhang1, Fiona Grennan-Jones1, Carol Lane2, D Aled Rees1, Colin Dayan1 & Marian Ludgate1
1 School of Medicine, Centre for Endocrine and Diabetes Sciences, Cardiff University, Cardiff, UK; 2 Department of Ophthalmology, Cardiff and Vale University Health Board, Heath Park, Cardiff, UK.

Expansion of the orbital contents, by adipogenesis and hyaluronan (HA) overproduction, causes Graves orbitopathy (GO). Orbital HA is generated predominantly by hyaluronan synthase 2 (HAS2), whose transcription is positively regulated by pAkt in cell lines. GO is most frequently associated with Graves’ disease (GD) in which thyroid stimulating antibodies bind the TSH receptor (TSHR) increasing cAMP. Previous studies highlight a role for TSAB and ‘neutral’ TSHR antibodies in GO, suggesting the involvement of additional TSHR or other signalling cascades e.g. IGF1 via PI3K. Furthermore the effects of TSHR activation may vary with its expression level and orbital fat (neural crest derived) may exhibit depot specific differences relevant to GO.

We have investigated using in vitro models to assess adipogenesis and HA production in preadipocyte/fibroblasts from orbital (n=10) and subcutaneous (n=10) adipose tissue in response to TSHR (activating mutation) and IGF1R activation (IGF1 addition).

In orbital preadipocytes adipogenesis significantly increased (40-60%) HAS2 transcripts (but decreased HAS1 and HAS3) and secreted HA (P<0.002). In contrast, subcutaneous preadipocyte adipogenesis significantly decreased (50-75%) all three HAS isoforms and secreted HA (P>0.03). Combining TSHR and IGF1R activation did not induce spontaneous adipogenesis but produced a synergistic effect on HAS2 transcription and HA generation in both depots. Surprisingly IGF1 treatment alone, either chronic or acute, had no effect on HAS2 transcription, despite significantly increasing the phospho:total Akt ratio; a pAkt inhibitor increased orbital HAS2 transcription. A stimulating effect of IGF1 on HAS2 transcription can be revealed by rapamycin in subcutaneous but a MEK inhibitor in orbital preadipocytes.

We conclude that a single physiological process (adipogenesis) in the orbit produces both mechanisms causing GO. Depot specific variations in HAS2 transcriptional control (feedback inhibition by mTOR or MEK in subcutaneous and orbit respectively) provide an explanation for these differences and the pathogenesis of GO.

P335
The use of tandem mass spectrometry to identify binding partners of PBF in thyroid cells
Neil Sharma, Robert Seed, Martin Read, Vicki Smith, Gregory Lewy, Gavin Ryan, Jim Fong, Perkin Kwan, Kristien Boelaert, Jayne Franklyn, Ashley Martin & Christopher McCabe
University of Birmingham, Birmingham, UK.

PBF is a proto-oncogene implicated in the aetiology of thyroid cancer. PBF binds to several proteins, including PITG and NIS, but its full function is unknown.

Understanding the range of interactions PBF has with thyroid cells is therefore vital; these were explored by tandem mass spectrometry (MS/MS), which relies on the predictable fragmentation pattern of an amino acid chain to identify the proteins present in a sample. PBF and TPC1 papillary thyroid cancer (PTC) cells transfected with HA-tagged PBF were lysed and immunoprecipitated with anti-HA and passed through MS/MS (n=4). 2587 proteins were identified. Those appearing in vector-only controls were eliminated, as were those identified by only 1 peptide in a single run.

Ten proteins were classed as being most likely to interact with PBF. Lyn, a Src tyrosine kinase, was identified by 2 peptides in the PTC1 immunoprecipitant but not the K1. This was supported by western blotting, where K1 cells were shown to have a low expression of Lyn compared to TPC1 cells. Contactin, a substrate of Src, was identified by 8 peptides, as were FAK2 (2 peptides, 2 runs) and the serine/threonine kinases cMos (1 peptide, 3 runs) and SMG (9 peptides). Given that we have recently identified PBF as a phosphorylated protein, interaction with Lyn, cMos and SMG may thus be critical to PBF phosphorylation. Binding to Familial adenomatous polyposis (FAP) protein (9 peptides) and ERC1 (2 peptides) was also apparent. There is a well documented link between FAP and thyroid cancer and an ERC1/ret mutation is able to induce PTC. Additionally identified proteins were GIT (3 peptides, 2 runs), ARAP (1 peptide, 2 runs), UACA (2 peptides) and ADAMTS18 (2 peptides). Future work will seek to validate these interactions using pull-down assays and co-immuno- precipitation, and to evaluate their function alongside PBF in thyroid cancer.

P336
Variation in levels of skewed X chromosome inactivation represents a shared pathogenic pathway for the common autoimmune thyroid diseases
Matthew Simmonds1, Oliver Brand1, Paul Newby2, Laura Jackson2, Chantal Hargreaves3, Jackie Carr-Smith4, Jayne Franklyn5 & Stephen Gough1
1 University of Oxford, Oxford, UK; 2 University of Birmingham, Birmingham, UK.

Graves’ disease (GD) and Hashimotos’ thyroiditis (HT), two of the most common autoimmune thyroid diseases (AITD), are caused by both shared and disease specific genetic determinants. In females one copy of the X chromosome is randomly inactivated, and although inactivation should occur with a parent of origin ratio of 50:50, skewed X chromosome inactivation (XCI) can occur, whereby >80% of a specific copy of the X chromosome is inactivated. Increased skewed XCI in GD is believed to explain the strong female preponderance seen.

Using data taken from all known GD XCI studies (5 studies totalling 454 GD and 610 controls), we performed meta-analysis which supported a role for skewed XCI in GD P=1.61×10^{-8}, OR=2.56 (95% CI=1.83–3.57). Some evidence for a role of skewed XCI in HT has previously been reported, but from relatively small datasets. We aimed to confirm these findings using microsatellite marker genotyping within the androgen receptor to determine XCI levels in a large UK Caucasian HT cohort consisting of 490 informative female HT patients and 325 female controls. All subjects gave informed written consent and the project was approved by the local ethics committee. Even using the largest HT dataset investigated to date only a trend towards increased skewed XCI (P=0.079) was found in our HT patients. Clinical correlations revealed that skewed XCI was more frequent in thyroid autoantibody negative HT patients than autoantibody positive patients (27.6 vs 13.6%), suggesting XCI skewing does not contribute to thyroid autoantibody production. However, meta-analysis of the current data and others (five studies totalling 671 HT cases and 592 controls) produced an overall significant pooled P=2.39×10^{-8} OR=1.93 (95% CI=1.41–2.64), confirming a role for XCI in HT. These and other ongoing studies determining how skewed XCI contributes to GD and HT will increase our understanding of this important shared AITD pathogenic pathway.

P337
Seasonal variation in thyroid autoimmunity as assessed by anti-thyroid peroxidase antibodies is related to temperature
Gina Middleton1, John Barker2 & Salman Razvi1,2
1 Newcastle University, Gateshead, Tyne and Wear, UK; 2 Gateshead Health NHS Foundation Trust, Gateshead, Tyne and Wear, UK.

Background
Environmental factors play a role in the pathogenesis of autoimmune conditions. The incidence of type 1 diabetes is higher in winter. It is unclear whether

Endocrine Abstracts (2011) Vol 25
autoimmune thyroid disease is similarly affected by seasonal variation. We aimed to study the variation in anti thyroid peroxidase (TPO) antibodies in relation to calendar month of sampling.

Methods
We obtained TPO-antibody results for the 12 months (October 2009 till September 2010). Individuals with known thyroid disease were excluded. TPO-antibody levels were measured quantitatively by ELISA and levels >50 IU/ml were classed as high. Geometric mean TPO-antibody concentrations were compared against month of sampling after correcting for multiple comparisons. Mean maximum temperatures and sunshine hours for the north east of England for the same time period were obtained from the Met Office. Stepwise linear regression analyses were performed to determine factors predicting TPO levels.

Results
The mean age (±S.D.) of the cohort (n=841) was 51.2 (21.7) years and majority were female (73.1%). The age and gender distribution of the cohort was similar in each month of sampling. Geometric mean (range) TPO-antibody levels were lower in winter than in summer with the lowest level in December of 15.25 (3–540) IU/ml and highest in August of 32.6 (9–3000) IU/ml (P<0.001). The proportion of individuals with high TPO levels was higher in August (32.9%) than in December (18.6%). Age, gender and maximum temperature on the day of sampling explained 6% of the variation in TPO levels with temperature alone accounting for 3% of variation.

Conclusions
Thyroid autoimmunity as measured by TPO-antibody levels has a seasonal variation which is lower in summer and higher in winter which is partly explained by the effect of temperature. This apparent association of thyroid autoimmunity with temperature may have implications for the future, especially in the context of global warming.

P339
Thyroid hormone receptor alpha is a permissive factor that regulates osteoclastogenesis indirectly
Jonathan J Nicholls, Charlotte E Combs, Graham R Williams & J H Duncan Bassett
Molecular Endocrinology Group, Department of Medicine and MRC Clinical Sciences Centre, Imperial College London, Hammersmith Hospital, London, UK.

Thyrotoxicosis is characterised by increased osteoclast activity. Thyroid hormone receptor alpha (TRα) is the predominant TR in mature osteoclasts and TRβ2 lacking TRα have skeletal hypothyroidism with impaired osteoclastic bone resorption. By contrast, mice lacking TRβ1 have skeletal hyperthyroidism and increased bone resorption. Thus, we hypothesized that TRβ1 acts via TRα to stimulate osteoclastogenesis. Osteoclasts were differentiated in vitro from wild-type (WT), TRα-null and TRβ2-null bone marrow precursors using M-CSF and RANKL in the absence of presence of TRβ1 and total RNA was isolated after 1 and 5 days of culture. Numbers of multinucleated tartrate-resistant acid phosphatase (TRAP) positive osteoclasts were quantified and TRAP activity determined at day 5. Data were analyzed by ANOVA to determine the effect of genotype on osteoclast parameters. The expression of genes involved in thyroid hormone signalling was investigated by RT-PCR. Precursor cells and mature osteoclasts expressed monocarboxylate transporters 8 and 10 (Mt8, Mt10), the type 2 and type 3 deiodinases (Dio2, Dio3) and TRβ1, TRβ2 and TRα1. Despite expression of these key genes, TRα-treatment did not affect WT osteoclast numbers (osteoclasts/field 4.3 ± 0.84 vs 3.0 ± 0.78, control versus TRα-treatment (n=6)) or differentiation (TRAP activity (mU/ml) 2.8 ± 0.7 vs 2.5 ± 0.7, control versus TRα-treatment (n=9)). Similarly, osteoclast cultures from TRα-null and TRβ2-null mice were unaltered by TRα-treatment. These data demonstrate that TRβ1 does not regulate osteoclastogenesis directly. Never-theless, increased numbers of mature osteoclasts were generated from TRα-null bone marrow cultures (osteoclasts/field 4.6 ± 0.9 vs 9.3 ± 2.2 vs 2.5 ± 0.7, WT versus TRα-null versus TRβ2-null, P<0.05 WT versus TRα-null). TRβ2-treatment (0.7 ± 0.05 WT versus TRβ2-null (n=4)) and TRAP activity increased 2.5-fold (TRAP activity (mU/ml) 4.4 ± 1.1 vs 11.7 ± 3.6 vs 4.5 ± 2.0, WT versus TRα-null versus TRβ2-null, P<0.05 WT versus TRβ2-null (n=4)). Together with the finding that TRα-null mice have fewer osteoclasts and reduced bone resorption, these data indicate that TRα plays a key permissive role to regulate osteoclastogenesis in vivo.

P338
A case of Graves’ disease associated with severe hypercalcaemia
Sarah Ali, Katie Wynne & Karim Meenan
Endocrine Unit, Imperial College Healthcare NHS Trust, London, UK.

A 25-year lady was referred by her GP with a six-month history of symptoms suggestive of thyrotoxicosis: weight loss, palpitations, heat intolerance and lethargy. She also complained of thirst, polyuria and nocturia. Examination revealed tachycardia, tremor and a moderately enlarged goitre which was partly explained by the effect of temperature. This apparent association of thyroid autoimmunity with temperature may have implications for the future, especially in the context of global warming.

In clinic, blood tests performed confirmed thyrotoxicosis: TSH <0.05 mU/l (NR 0.3–4.2 mU/l), free T 3 >46.1 pmol/l (NR 2.5–5.7 pmol/l) and free T 4 >69.1 pmol/l (NR 9.0–26.0 pmol/l). Serum anti-TSH receptor antibody was positive (>30.0 u/ml, NR 0.0–4.0 u/ml). Interestingly, corrected calcium was significantly raised at 3.35 mmol/l (NR 2.15–2.60 mmol/l), phosphate was 1.50 mmol/l (NR 0.8–1.4 mmol/l) and parathyroid hormone was non-suppressed but low-normal: 2.1 pmol/l (NR 1.1–6.8 pmol/l). Alkaline phosphate was normal and other causes of hypercalcaemia were excluded. She was admitted for treatment of the hypercalcaemia with aggressive intravenous fluids. Carbimazole 20 mg tds and propranolol 40 mg tds were concurrently started. She was discharged home once the corrected calcium fell to 2.79 mmol/l and was closely followed up in the outpatient setting. Following aggressive treatment of the Graves’ disease, biochemical results were significantly better two weeks later, including resolution of the hypercalcaemia (TSH <0.05 mU/l, T 3 16.0 pmol/l, T 4 4.2 pmol/l, corrected Ca 2.14 mmol/l, phosphate 0.87 mmol/l, PTH 2.5 pmol/l). This severe hypercalcaemia was therefore attributed to thyrotoxicosis as opposed to primary hyperparathyroidism. Interestingly, this lady was seen in clinic two months later and due to five weeks of noncompliance with Carbimazole, she was once again thyrotoxic and hypercalcaemic.

Conclusions
Thyrotoxicosis is commonly associated with abnormalities in bone metabolism, however the resultant hypercalcaemia is usually mild and asymptomatic. If hypercalcaemia is severe and symptomatic, there is often an alternative cause. In support of severe hypercalcaemia due to thyrotoxicosis is the reversal of hypercalcaemia with treatment of the hyperthyroidism. This case is interesting as thyrotoxicosis with such marked hypercalcaemia is uncommon and a corrected calcium of 3.35 mmol/l appears to be one of the most significantly elevated levels reported.

P340
Targeted hPTTG overexpression in vivo induces reduced cellular proliferation and p53 stabilisation resulting in decreased thyroid size
Gavin Ryan, Gregory Lewy, Martin Read, Robert Seed, Neil Sharma, Vicki Smith, Jim Fong, Perkin Kwan, Jayne Franklyn, Christopher McCabe & Kristien Boelaert
University of Birmingham, Birmingham, UK.

The human pituitary tumor transforming gene (hPTTG) plays important roles in tumourigenesis through its function as a securin and by interaction with the tumour suppressor gene p53. We have previously identified hPTTG expression as a prognostic indicator for differentiated thyroid cancer. We recently generated a transgenic mouse model overexpressing hPTTG targeted to the thyroid gland via a thyroglobulin promoter to delineate PTTG effects on thyroid tumourigenesis in vivo. Interestingly, following screening of 92 pups (n = 121), from heterozygote hPTTG mice matings, no homozygote mice were identified. Furthermore, hPTTG mouse litter sizes were significantly reduced compared with WT litters (n = 6.83 vs n = 9.35, P = 0.001), suggesting homozygous thyroidal PTTG overexpression may be embryonically lethal. At 6 weeks transgenic mice exhibited significantly reduced thyroid weights (0.86-fold, P = 0.005, n = 36) when compared with age and gender matched WT mice. To determine if this reduction in size was due to lower cellular proliferation rates we assessed PCNA and cyclin D1 expression in excised thyroids. Cyclin D1 levels, measured by immunohistochemistry, were significantly reduced (0.59-fold, P = 0.015) while PCNA, measured by western blotting, showed a non-significant reduction in expression (0.61-fold, P = 0.07) in transgenic mice. Expression of p53, an important gene in DNA repair and cell cycle regulation, was significantly increased at both mRNA (1.7-fold, P = 0.02) and protein level (2.1-fold, P = 0.02) in IPTTG mice. Furthermore expression of p21 mRNA, an effector of p53 action and an inhibitor of cyclin-dependent kinases and PCNA expression was significantly increased in transgenic mouse thyroids (1.6-fold, P = 0.02). In vitro experiments confirmed significantly increased p21 mRNA expression (1.9-fold, P = 0.01) following hPTTG transfection in the K1 human papillary thyroid cancer cell line. In conclusion, targeted thyroidal hPTTG overexpression results in reduced cellular proliferation and upregulation of p53 and p21, suggesting activation of DNA damage repair pathways and apoptosis.
P341
What is the influence of rheumatologic conditions on response to medical therapy of Graves' disease?
Kavita Gulati1,2, Miriam O'Sullivan3, Michael Molloy2, Mary Stapleton4, Sinead Harron5, John O'Halloran1, John Ryan4 & Antoinette Tuthill1
1Cork University Hospital, Cork, Ireland; 2University College Cork, Cork, Ireland.

Anecdotal reports suggest that management of Graves’ disease (GD) is more challenging in patients with concomitant autoimmune disease, however there are few data available to support this premise and thus no satisfactory evidence base to guide clinicians. We aimed to identify the impact of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) on treatment outcomes of GD.

We identified all GD patients (n=265) who attended endocrinology clinics at a large tertiary referral hospital (1994–2001). Patients with GD and either RA or SLE (n=16) were selected as cases and matched by age and gender to patients with GD alone (n=48) and those with RA or SLE alone (n=48). Patients were excluded as controls if they had another autoimmune disease or treatment with steroid therapy >3 months. Factors assessed included patient demographics, immunologic markers and treatment outcomes.

Patients with GD were predominantly female (86%) with a mean age of 50 years (range 16–96 yrs). 14% of patients with GD had another autoimmune disease, the commonest being RA. 44% of patients suffered from Graves’ ophthalmopathy with significantly (P=0.04) increased risk of ophthalmopathy in smokers. We found that there were no differences in response to initial anti-thyroid drug therapy between patients with GD alone and those with GD+RA/SLE (P=0.75). However, patients with GD+RA/SLE relapsed much more quickly after 1st treatment (mean 6.2±5.8 months) compared to the GD alone group (mean 22.48±37.2 months) (P=0.034).

A greater proportion of patients with GD alone underwent radioactive iodine therapy (45.8%) than in the GD+RA/SLE group (13%) (P=0.03). This was partially explained by difference in rates of Graves’ ophthalmopathy between groups.

We found that concomitant RA or SLE did not alter initial response rates to anti-thyroid medication, although this group had significantly higher relapse rates, suggesting that close monitoring for relapse is required even when the patient has achieved euthyroid state.

P342
Hashimoto’s thyroiditis with associated neurological deficits (Hashimoto’s encephalopathy)
Elin Owen, Stella Woodward, Avril Wayte, Sarah Wenham & Antony Wilton
University of Wales, Bangor, UK.

A 48-year-old female received radioiodine ablation therapy for thyrotoxicosis secondary to a solitary toxic nodule. The subsequent unexpected requirement for replacement thyroid hormone was explained by Hashimoto’s thyroiditis with an anti-thyroid peroxidase antibody level of 692.3 IU/ml. Thirty months later she presented with debilitating neurological deficits which responded to treatment with steroid therapy (mean 6.2±5.8 months) and thus no satisfactory evidence base to guide clinicians. We aimed to identify the impact of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) on treatment outcomes of GD.

We identified all GD patients (n=265) who attended endocrinology clinics at a large tertiary referral hospital (1994–2001). Patients with GD and either RA or SLE (n=16) were selected as cases and matched by age and gender to patients with GD alone (n=48) and those with RA or SLE alone (n=48). Patients were excluded as controls if they had another autoimmune disease or treatment with steroid therapy >3 months. Factors assessed included patient demographics, immunologic markers and treatment outcomes.

Patients with GD were predominantly female (86%) with a mean age of 50 years (range 16–96 yrs). 14% of patients with GD had another autoimmune disease, the commonest being RA. 44% of patients suffered from Graves’ ophthalmopathy with significantly (P=0.04) increased risk of ophthalmopathy in smokers. We found that there were no differences in response to initial anti-thyroid drug therapy between patients with GD alone and those with GD+RA/SLE (P=0.75). However, patients with GD+RA/SLE relapsed much more quickly after 1st treatment (mean 6.2±5.8 months) compared to the GD alone group (mean 22.48±37.2 months) (P=0.034).

A greater proportion of patients with GD alone underwent radioactive iodine therapy (45.8%) than in the GD+RA/SLE group (13%) (P=0.03). This was partially explained by difference in rates of Graves’ ophthalmopathy between groups.

We found that concomitant RA or SLE did not alter initial response rates to anti-thyroid medication, although this group had significantly higher relapse rates, suggesting that close monitoring for relapse is required even when the patient has achieved euthyroid state.

P343
Optimal use of thyroid antibody assays in the identification of auto-immune thyroid disease
Dulmini Kariyawasam1, Lingling Chuah1, Swana Granville2, Yousof Karimi3 & Paul Carroll4
1Department of Endocrinology, Guy’s and St. Thomas’ NHS Foundation Trust, London, UK; 2Department of Immunology, Guy’s and St. Thomas’ NHS Foundation Trust, London, UK.

Background
A variety of thyroid antibody assays are used in the diagnosis of auto-immune thyroid disease (AITD). Commonly both thyroid peroxidase (TPOab) and thyroglobulin antibodies (TGab) are measured but the added value of testing two markers has not been established.

Method
We retrospectively collected clinical and laboratory data on 500 consecutive patients who had thyroid autoantibodies requested from a specialist endocrine department of a tertiary hospital from December 2008 to October 2010. TPOab and TGab were simultaneously analysed using the FIDMS multiplex bead assay (BMD, Marne La Vallée, France).

Results
There were 399 (79.8%) females and 101 (20.2%) males in the cohort, aged 43.5±15.3 (mean±SD) years. 163 (32.6%) patients had Graves’ disease and 118 (23.6%) had Hashimoto’s thyroiditis. The other diagnoses included: thyroid nodules 101 (20.2%), other autoimmune diseases e.g. type 1 diabetes 58 (11.6%), primary hypothyroidism 41 (8.2%) and transient thyroiditis 19 (3.8%). From the 163 patients with Graves’ disease 107 (65.6%) had TPOab, 64 (39.3%) had TGab. Of the 118 patients with Hashimoto’s thyroiditis, 103 (87.3%) were positive for TPOab and 73 (61.9%) positive for TGab.

Conclusion
TPOab testing was superior to TGab assay in identifying patients with both Graves’ disease and Hashimoto’s thyroiditis. Although there may be a rationale in reserving TGab testing as a second-line test in patients testing negative for TPOab, this would miss 8.6% of Graves’ and 11.9% of Hashimoto’s thyroiditis patients. The multiplex assay tests both antibodies concurrently and dual testing provides increased sensitivity for AITD. The higher cost of the multiplex assay would be offset by the need to test TGab separately in the anti-TPO negative patients, which constitute 34.4% of Graves’ disease and 12.7% of Hashimoto’s patients.

P344
Thyroid hormone changes and psychological response to high altitude stress: effect of ethnicity
Meenakshi Sachidanandam1, Suresh Arumugam2 & UdaySankar Ray1
1Defence Institute of Physiology and Allied Sciences, Delhi, India; 2Defence Institute of Psychological Research, Delhi, India.

Objective
Acclimatization to high-altitude is a dynamic process affecting the neuro-endocrine and physiological systems. Psychological well-being is also influenced by hypobaric-hypoxia. Psychiatric stress is known to influence the hypothalamo-pituitary-adrenal/thyroid axis. However, the involvement/contribution of ‘psychological-performance’ on hormone changes at high-altitude is not known. The objective of this study was to examine: i) the effect of ethnicity on cortisol, thyroid hormones and psychological performance during high-altitude exposure in lowlanders as compared to sea-level, and with high-altitude-natives (HAN) at high-altitude; ii) if there is any relation between psychological variables and hormones at high-altitude in the Indian population.

Methods
Male volunteers (20–50 years) belonging to Caucasian/Indo-European (n = 25), Mongoloid/Tibeto-Burman (n = 31) and Australoid/Dravidian (n = 28) ethnicities were studied at sea-level and after 3–4 weeks of stay at ~4500 m. High-altitude-naïve (HAN, n = 21) were studied at ~4500 m only. Plasma hormone (EIA/ELISA)/biochemical estimation, physiological and psychological evaluation was conducted on all subjects. Significance at P < 0.05.
Results
In lowlanders, there was a significant change in loneliness, fear-of-death; thyroid hormones at high-altitude. Interactive effect between high-altitude × ethnicity was observed for psychological and hormone variables (except hopelessness, freeT₃). Significant variation in hormones and psychological variables was observed between lowlanders and HAN at high-altitude (except memory, TSH). Significant correlation was observed between fear-of-death/fear of loneliness for Caucasoids (r = 0.55) and Mongoloids (r = 0.49); loneliness/freeT₄ (r = 0.37) and fear-of-death/freeT₄ (r = 0.36) for Mongoloids.

Conclusion
The circulating adiponectin and leptin are affected by thyroid hormone levels. Adiposity could be the metabolic link between thyroid status and adipocytokines.

P346
Changes in urinary fractional excretion (FE) of calcium and phosphate following treatment of hyperthyroid cats
Tim Williams, Jonathan Elliott & Harriet Syme
Royal Veterinary College, London, UK.

Hypothyroid cats have elevated parathyroid hormone (PTH) concentrations and suppressed fibroblast growth factor-23 (FGF-23) concentrations, both of which normalise following treatment of hyperthyroidism. PTH, FGF-23, and thyroid hormone can influence the renal reabsorption of calcium and phosphate. The aim of the present study was to assess the influence of hyperthyroidism on renal tubular function in cats indirectly, by comparing the FE of electrolytes in hyperthyroid cats with and without chronic kidney disease (CKD), which could also affect the FE of electrolytes, before and after treatment of hyperthyroidism. Hyperthyroid cats were treated for hyperthyroidism and monitored for the development of azotaemia over a 240-day follow up period. Blood and urine samples were collected at baseline and following treatment and the FE of calcium and phosphorus calculated. The Wilcoxon signed rank test was used to compare FE before and after treatment. Data are presented as median (25th, 75th percentile).

Forty-two hyperthyroid cats (27 non-azotaemic and 15 azotaemic) were included in the study. In the non-azotaemic cats, there was an increase in the FE of calcium (0.10 (0.07, 0.15) vs 0.14 (0.10, 0.29) %; P = 0.001) and phosphate following treatment (37.2 (19.8, 42.3) vs 51.9 (34.8, 63.0) %; P = 0.001). Following treatment of azotaemic cats, there was an increase in the FE of calcium (0.24 (0.11, 0.49) vs 0.77 (0.19, 1.31) %; P = 0.005), however the FE of phosphate did not increase significantly (48.1 (37.7, 58.9) vs 61.2 (47.6, 80.9) %; P = 0.156). FE of calcium and phosphate increased following treatment of hyperthyroidism, however the non-significant increase in FE of phosphate following treatment of azotaemic cats might indicate that the maximum level of phosphate excretion was reached in some animals.

P347
Retrospective analysis of thyroid fine needle aspiration (FNA) in a tertiary hospital
Alia Nasir, Subash Sivaraman, Hala Alsaifadi, Steve Ferryman, Paul Mathews, Yen Chang Yeo, Tom Goodfellow, Nick Hedley, Kim Brett, Barbara Smith, Hisham Mehaan, Martin OWickert & Sajid Sankar
Wisdom Centre, Coventry and Warwickshire, Coventry, UK.

Aim
In this study we have compared the adequacy rates of FNA samples between more experienced and less experienced operators in thyroid FNA.

Introduction
The prevalence of thyroid nodules is estimated to be about 4–7%, increasing up to 50% in the adult population. US guided FNA has become the preliminary investigation of choice for thyroid nodules, as it is widely available and has been proven to increase diagnostic efficacy. In 2004, NICE recommended the setup of ‘one-stop clinics’ for patients with neck lumps.

Material and methods
Data was collected retrospectively from 180 patients who underwent ultrasound scan (US) FNA of thyroid over a period of 21 months, from January 2008 to October 2009; either through One-stop clinic or routine referrals from clinics. Results were analysed using SPSS version 16.

Results
Of 312 FNA were performed in 180 (156 females) patients, 143 were reported as inadequate sample/indeterminate.

Logistic regression analysis using diagnostic (Thy 2–5) versus non-diagnostic (Thy1) samples as the dependent variable and age, sex, TSH and operator as covariates showed a significantly higher rate of Thy1 samples when FNA was performed by less experienced operators (59 vs 39%, P = 0.031), although only 5% of the variation was explained by the total model. FNAC had sensitivity of 72.2% and specificity of 55.9% when compared with histopathology in the 58 patients who underwent thyroidectomy. Positive

Endocrine Abstracts (2011) Vol 25
Obesity is a global health concern and the proportion of overweight and obese people in the UK is rapidly increasing. Patients undergoing treatment for hyperthyroidism frequently express concerns regarding excessive weight gain especially when offered treatment with¹³¹I. We followed 1159 patients with overt hyperthyroidism to determine the extent of weight changes and to identify risk factors for weight gain following treatment. Overall, BMI remained unchanged in 135 (11.7%), reduced in 68 (5.9%) and increased in 956 (82.4%). Mean weight gain was 8.42 ± 2.0 kg and increase in BMI was 3.15 ± 0.07, over a mean 26.55 ± 0.61 months. At presentation, 29.9% of patients were overweight (BMI ≥ 25) and this ratio rose to 38.1% at the end of follow-up (P < 0.001). The proportion of obese patients (BMI ≥ 30) increased from 17.2 to 34.9% (P < 0.001). The reporting of symptoms of prior weight loss (n = 724, AOR: 1.88, P < 0.001), male gender (n = 276, AOR: 1.50, P = 0.04), an underlying aetiology of Graves’ disease (n = 446, AOR: 1.41, P = 0.04), and higher FT4 concentration at presentation (AOR: 1:01 per 1 pmol/l increase, P < 0.001) were associated with increased risk of weight gain, whereas the presence of obesity (n = 164, AOR: 0.62, P = 0.02) reduced this probability. The proportion of patients gaining weight was similar in 332 subjects treated with thionamides only when compared with 827 receiving ¹³¹I (weight gain in 79.1 vs 84.7%, P = 0.02) reduced this probability. The proportion of patients gaining weight was similar when compared with those not becoming hypothyroid (84.7 vs 79.1%, P = 0.08). Multivariate analyses identified male gender (AOR: 1.48, P = 0.05), symptoms of prior weight loss (AOR: 1.66, P = 0.003) and higher presenting FT4 (AOR: 1.01, P = 0.008) as independent predictors of weight gain.

Conclusion
Treatment of hyperthyroidism is associated with marked weight gain and increased risks of becoming overweight or obese. Males, subjects with more severe hyperthyroidism and those with prior weight loss are particularly at risk of gaining weight. Neither the administration of ¹³¹I nor the development of hyperthyroidism subsequently, are associated with increased probabilities of gaining weight.

Symptoms include visual distortion or discomfort (often ameliorated by squinting), monocular diplopia or ‘ghosting’ and photosensitivity. The reduced tear production and rubbing of eyes, common in Graves’ disease, is a known precipitant of keratoconus. There is an increased prevalence of keratoconus in immune disorders, including hashimoto’s thyroiditis (OR 2 (CI 1.2–3.3)) (1). In patients with keratoconus the normal thyroxine level in tears has been found to be two orders of magnitude lower than in the serum. Interestingly, independently of their serum thyroid function, patients with keratoconus have tear thyroxine levels 2–50 fold higher than subjects free of ocular pathologies (2). Indeed tear thyroxine levels are higher during progression of keratoconus and decline once corneal curvature reaches a new steady value (2).

References
P352
Variation in free T3 (fT3) within the reference range is associated with bone development in children, and mediated via alteration in body mass indices
Peter Taylor¹, Adrian Sayers², Ahmed Iqbal³, Andrew Taylor³, Colin Dayan² & Jonathan Tobias¹
¹Henry Wellcome Laboratories for Integrative Neurosciences and Endocrinology, University of Bristol, Bristol, UK; ²Academic Rheumatology, Avon Orthopaedic Centre, Southmead Hospital, Bristol, UK; ³Department of Medicine, Centre for Endocrine and Diabetes Sciences, Cardiff University School of Medicine, Cardiff, UK.

Objective
The hypothalamus–pituitary–thyroid axis profoundly influences skeletal development. Whilst the adverse effects of thyroid dysfunction on bone are well established; the effects of variation within the normal range is less clear.

Design
In a subset of the Avon Longitudinal Study of Parents and Children we performed full thyroid function tests from stored samples taken at age 7. 668 children had thyroid hormone parameters within the reference range. They underwent a total body DXA scan aged 9 to analyse body mass indices, bone mineral density (BMD), bone area (BA), bone mineral content (BMC) and BMC adjusted for area (aBMC). At age 15, 460 children underwent repeat total body DXA.

Results
A subset of the Avon Longitudinal Study of Parents and Children was included in the analysis. Free T3 was associated with fat mass at age 15 (P≤0.0001). No associations were seen with TSH and fT4 levels on body mass indices or bone outcomes. Conclusion
Variation within the reference range in fT3, but not fT4 or TSH was strongly associated with body mass indices, and BMC. The BMC association was via BA, not BMD as no association was observed with aBMC. The associations between fT3 and bone were lost when adjusted for body mass indices highlighting that this association is mediated through them. Surprisingly there was a positive association between fT3 and fat mass consistent at ages 9 and 15; likely due to an unexplained mechanism between fT3 and childhood adiposity rather than a direct effect between fT3 and fat mass.

P354
Generalised anxiety disorder following thyroidectomy
Eleftheria Panteliou & Khash Nikooka
King George’s Hospital, BHR University Hospitals, London, UK.

A 38-year-old woman presented with tremor and anxiety 3 weeks into her pregnancy and was diagnosed with thyrotoxicosis. One year postpartum, the thyroid appeared multinodular with a dominant nodule that showed deficient uptake on the technetium scan. Her thyroid function was normal and thyroid peroxidase antibodies were negative. She developed an enlarging goitre and underwent total thyroidectomy following which she developed persistent hypocalcaemia and hypomagnesaemia.

She presented with similar to her gestational symptoms, 3 months later. Her free T4 was 25 pmol/l and her symptoms were attributed to metabolic causes. Despite normal thyroid profile and electrolytes her symptoms deteriorated. A psychiatric review concluded that her symptoms were associated to a generalised anxiety disorder.

Thyroid has an important effect on cognition and mood. Hypothyroidism is associated with depression that can improve with treatment although full
Neuropsychiatric symptoms can persist in thyrotoxicosis despite maintaining a euthyroid state. Up to 7% of women can develop postpartum thyroid dysfunction. Antibodies against thyroid peroxidase can be a marker of postpartum depression. Post-operative cognitive dysfunction is common in advanced age and possibly caused by cerebral microemboli. Although neuropsychiatric changes following major surgery are known, there is not a well established association between anxiety disorders and minor surgery. Generalised anxiety disorder is associated with headache, paraesthesia, insomnia, tremor, fatigue, difficulty in concentration, sweating, palpitations, dyspnoea, nausea, urinary frequency, body pains and anxiety. It results from a combination of genetic propensity and stressful environmental conditions that can alter the function in amygdala and cingulated gyrus and increase the cortisol levels and sympathetic activity. Some studies support that the persistence of neuropsychiatric symptoms, despite the withdrawal of the triggering event, can be associated with permanent changes in the brain. Cases like this should potentiate our vigilance in the early recognition and management of such symptoms.
Author Index

Abara, A P88
Abass, M P161
Abayasekara, R P113
Abbas, M P117
Abbas, M P349 & P350
Abbasakoor, N P99
Abd El Baki, R P160, P161, P162 & P280
Abdelsalam, A P151
Abdelhalim, M P117
Abdelhalim, M P106, P107, P151, P153 & P157
Abouda, G P351
Abouglila, K P64
Abraham, P OC3.8
Abu-Shady, M P107 & P157
Achermann, J CM4.4
Achilleos, K P61
Adams, V P150
Adamson, A P244
Adesina, O P52
Adham, M P31
Aditya, BS P81
Adlan, M P76
Aflorei, D P282
Afolabi, PR P146
Agustsson, T P37
Ahasan, M OC2.1
Ahassan, M P118
Ahlquist, J P61
Ahmad, J P128
Ahmed, A P221 & P311
Ajala, O P228
Ajanaku, A P251
Al-Akbar, AA P329
Al-akbar, ANBAA P20
Al-Attas, O P124
Al-Da’ghri, N P124
Al-Dujaili, E P139 & P292
Al-jabouri, M P305
Al-Kutubi, H P358 & P59
Alatzoglou, K OC5.3
Alatzoglou, KS P259
Alberts, B P68
Albayyati, A P158
Albu, A P282
Alexander, S AP1.2
Alhazmi, J P312
Ali, A P269
Ali, MA P310
Ali, S P88, P212 & P338
Aljasser, S P31
Alkharfy, K P124
Allahabadia, A CM1.2
Allen, K P330
Alloio, B P14
Almond, K P206
Alokail, M P124
Alsaafari, H P347
Alzahrani, S OC3.7
Amin, A P203, P239 & P278
Andrews, R SIG2.3
Ang, AL P10 & P75
Angelin, B S6.4
Aniket, T P269
Annamalai, A OC3.1
Annamalai, AK P192 & P309
Arends, MJ P192
Arlt, W S4.3, CM2.3, OC1.6, OC1.7, OC2.5, P229 & P300
Armstrong, B P148
Arulselvan, S P5
Arumugam, S P344
Arvan, P P125
Ashawesh, K P20, P33 & P329
Ashford, M P112
Aspinall, S P86 & P182
Aspry, EC OC4.7
Atay, Z P301
Attin, S P126, P149, P274 & P326
Atkin, SL P7, P146 & P265
Attia, A-E-A P161
Aung, T P286
Awais, R P244
Aye, M P7, P326 & P351
Aye, MM P7 & P146
Aylwin, S P130 & P327
Ayuk, J CM3.4 & P148
Azenabor, A P120
Aziz, R P130
Baborie, A P232
Babur, M OC3.5 & P179
Bachuwar, R P325
Baculescu, N P282
Bailley, C OC3.2
Balley, M OC1.4
Bairisto, C P144
Baitharu, I P109
Baki, RA P345
Balaguruswamy, S P234
Baldeweg, S CG1.1
Ball, A P20
Ball, S P144
Banerjee, A P57
Bangar, V P21
Bannon, N OC3.8
Bano, G P240
Banu, Z S5.2
Barber, T P238
Barhwal, K P109
Barker, J P337
Barnard, M P10 & P75
Barnes, D P138
Bartalena, L CM1.3
Barth, J SIG3.4, P248 & P257
Bartke, A S4.1 & OC1.3
Barton, DM P216
Baskar, V P217
Bassett, D OC4.3 & P154
Bassett, E P114 & P115
Bassett, JHD S6.1, P8 & P339
Bastawrous, M P57
Battaglia, G P250
Baxter, J P272
Baxter, P P248 & P257
Bdiri, A P20, P33 & P329
Beale, K P132 & P272
Beales, P S5.1 & S5.2
Beber, T OC3.5 & P179
Becker, R OC3.8
Becker, K OC4.7
Beck, S S2.2
Beckett, G SIG3.3
Begley, J P48
Begum, G P179
Begum, S P177
Behara, A P2
Belchetza, P SE1.3
Bentley, E P296
Bentley, H P298
Bereket, A P301
Berney, D P187
Berry, A OC1.6 & P167
Bertagna, X P15 & MTE2
Bevan, JS PL9
Bewick, G P155
Bezakova, A P204
Bhadada, S P2 & P127
Bhansali, A P2, P50, P127 & P242
Bhansali, S P127
Bhattacharya, S OC5.4
Bienek, R P110
Bienkiewicz, M P268
Bikker, P P206
Billik, D P172
Billig, H P264
Bingham, E P298
Biswas, S P238
Bitner-GLindzicz, M P259
Black, D P18
Blackmore, A P101
Bloom, S P313, P132, P154, P155, P272, P278 & P279
Bloom, SR OC4.7 & P269
Boelaert, K OC3.4, OC3.8, P195, P198, P321, P335, P340 & P348
Boelen, A P8 & P154
Boonen, S P18
Boughton, C P278
Bouloux, P-M P105
Bowen-Jones, D P45
Bower, L P188
Bowles, S P22
Bowman, P P314
Boyle, A P8
Brabant, G OC3.5, P36
Brahma, A P71
Brand, O P336
Brass, A P295
Brett, K P347
Brewster, S P46
Brickley, G P140
Brooke, J P152
Brown, K PL1
Walter, D P152
Wampers, M P165
Wang, C SIG3.2
Wangnoo, S P128
Ward, A P330
Ward, E P39, P97 & P226
Warfield, A OC3.4 & P321
Wark, G OC3.1
Warlow, M P65
Warner, D P230
Wass, J CG1.1, P42, P68, P168, P174, P186, P211, P236, P238, P249, P286 & P307
Waterson, M P188
Watkinson, J P321
Watt, A OC5.4
Watt, P P140
Wayte, A P342
Weaver, J P180 & P201
Webb, D P231
Webber, L P158
Webster, S YE1.4
Weedon, M S1.4
Weickert, M O P347
Weigelin, B AP1.2
Weiss, A P284
Wells, T OC4.8 & P148
Wenham, S P342
Westwood, M P205
Whatmore, A P205
White, A P179
White, D P158
White, H P181
White, K P283
White, M P130
Williams, A P8
Williams, G OC1.1, OC3.8, OC4.3 & P154
Williams, GR PL6, P8 & P339
Williams, J OC3.5
Williams, K OC3.5 & P179
Williams, M P222
Williams, T P346
Wilmer, M P136
Wilton, A P342
van Winkel, R P165
Winocour, P P108
Winston, R P275
Winter, J P112
Withenshaw, N P34
Wong, C P150
Wong, S P310
Wong, S L P94
Wong, W P170
Wood, G P139
Wood, S OC2.4
Woodland, J P114
Woodroffe, N P159
Woods, D P86, P144, P182 & P319
Woods, K P259
Woodward, S P342
Wotton, SA P146
Wright, D N2.3
Wright, M P28 & P29
Wright, T OC4.5
Wu, F P79 & P285
Wynne, K P88, P279, P338, P349 & P350
Wyrwoll, C OC5.5
Yaacoub, G P67 & P189
Yang, C OC2.2
Yeo, YC P347
Yu, M P8
Yu, N OC1.2 & P13
van Zaane, B OC3.7
Zahir, ARM P214
Zaki, I P107, P151 & P157
Zatko, T P204
van Zeijl, CJ P8
Zeka, G P320
Zhang, L P334
Zielinszka, A P166
Take a new look at ...

Endocrine-Related Cancer

Need to know more?

The established global forum on hormones and cancer with a high 5-year impact factor of 5.683

Publishing basic, translational and clinical research on hormone-dependent cancers and cancers of endocrine organs

Reviews free online at www.try-erc.org

- Introducing the new Editor-in-Chief, Professor Charis Eng, Cleveland Clinic, USA
- Now publishing bi-monthly
- Continuous online publication of NEW ARTICLES
- Accepted pre-prints online in 24 hours

- Online with HighWire Press
- Easy online submission with ScholarOne
- An official journal of the European Society of Endocrinology

Sign up for free tables of contents alerting www.endocrinology-journals.org

Society for Endocrinology

Published by BioScientifica