Endocrine Abstracts

March 2014 Volume 34
ISSN 1479-6848 (online)

Society for Endocrinology BES 2014

24–27 March 2014, Liverpool, UK

published by
bioscientifica

Online version available at
www.endocrine-abstracts.org

Endocrine Abstracts

Volume 34
March 2014

SOCIETY FOR ENDOCRINOLOGY BES 2014

24–27 March 2014

The Arena & Convention Centre, Liverpool, UK

Abstract Book

EDITORS

The abstracts submitted were marked by the Abstract Marking panel selected by the Programme Committee

Programme Committee

C McCabe (Chair of the Programme)
M Druce (Programme Co-ordinator)
C McArdle (Programme Co-ordinator)

Members

A Toogood
A Logan
E Davies
G Lavery
J Davis
S Pearce
R Andrew
N Gittoes
W Farrell
Ni Kieffer
C Duncan
V Caberera-Sharpe
R Semple
L Crowne

Abstract Marking Panel

J Ahlquist Southend
A Allahbadia Sheffield
B Allolio Wurzburg, Germany
R Andrew Edinburgh
R Andrews Bristol
W Arlt Birmingham
S Atkin Hull
M Aye Hull
S Aylwin London
J Ayuk Birmingham
L Bailey Liverpool
J Barth Leeds
A Bates Birmingham
P Belchetz Wetherby
M Besser London
K Boelaert Birmingham
P Bouloux London
N Bridges London
T Carpenter Yale, USA
P Carroll London
K Chapman Edinburgh
K Chatterjee Cambridge
T Cheetham Newcastle
P Clayton Manchester
J Compston Cambridge
M Cooper Birmingham
S Cox South Devon
A Crown Brighton
M Dattani London
E Davies Glasgow
J Davis Manchester
C Dayan Cardiff
M DeBono Sheffield
W Dhillo London
K Docherty Abderdeen
W Drake London
M Druce London
C Duncan Edinburgh
B Evans Cardiff
W Farrell Birmingham
D Flynt Strathclyde
R Fowkes London
J Franklyn Birmingham
S Franks London
W Fraser Liverepool
M Freel Glasgow
N Gittoes Birmingham
H Gleeson Leicester
M Gurnell Cambridge
D Halsall Cambridge
P Hanson Newham
L Heisler Cambridge
S Hillier Edinburgh
M Holmes Edinburgh
J Howard London
T Howlett Leicester
S Hunter Belfast
A James Newcastle
C Johnston West Hertfordshire
K Jonas London
N Karavitaki Oxford
N Kieffer Leicester
P King London
S Kumar Warwick
G Lavery Birmingham
V Lawrence London
J Lenders Netherlands
A Levy Bristol
M Levy Leicester
A Lopez Bernal Bristol
S MacKenzie Glasgow
C A McArdle Bristol
P McTernan Warwick
D Morganstein London
D Morris Ipswich
J Munday Portsmouth
K Murphy London
R Murray Leeds
J Newell-Price Sheffield
S Orme Leeds
K Owen Oxford
S Pearce Newcastle
P Pickett Shrewsbury
R Quinton Newcastle
S Razvi Gateshead
M Read Birmingham
A Rees Cardiff
P Saunders Edinburgh
P Selby Manchester
D Shapiro Glasgow
L Shepherd Birmingham
C Shoulders London
H Simpson Cambridge
V Smith Birmingham
P Squires Warwick
C Stewart Liverpool
R Stimson Edinburgh
F Swords Norwich
T Tan London
C Thirlwell London
A Toft Edinburgh
J Tomlinson Birmingham
A Toogood Birmingham
P Trainer Manchester
B Vaidya Exeter
J Valle Manchester
M Vanderpump London
E Ward Leeds
G Wark Guildford
J Webster Sheffield
A Weetman Sheffield
M Westwood Machester

Corporate supporters
The Society for Endocrinology would like to thank its Corporate Supporters for their generous financial assistance.

Platinum Supporters
Bioscientifica
Ipsen Ltd

Gold Supporters
Almirall
Bayer Healthcare
Novartis Pharmaceuticals UK Ltd
Pfizer Ltd
Viropharma

Society for Endocrinology
22 Apex Court
Woodlands
Bradley Stoke
Bristol BS32 4JT, UK

Tel: +44 (0) 1454 642200
Fax: +44 (0) 1454 642220
E-mail: info@endocrinology.org
Website: http://www.endocrinology.org

Conference Secretariat
Bioscientifica Ltd
Euro House, 22 Apex Court
Woodlands
Bradley Stoke
Bristol BS32 4JT, UK

Contact: Claire Arrigoni/Harriet Edwards
Tel: +44 (0)1454 642240
Fax: +44 (0)1454 642222
E-mail: conferences@bioscientifica.com
Website: http://www.bioscientifica.com

CONTENTS

Plenary Lecturers' Biographical Notes

Society for Endocrinology Dale Medal Lecture 2013

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

http://www.endocrine-abstracts.org/ea/0031/ea0031pl1biog.htm

Society for Endocrinology Dale Medal Lecture 2014

Bert W O'Malley, Baylor College of Medicine, Houston, USA

Bert W O'Malley, MD is the Tom Thompson Distinguished Service Professor of Molecular and Cellular Biology at Baylor College of Medicine. A native of Pittsburgh, he has a bachelor's degree from the University of Pittsburgh (1959) and an MD from their School of Medicine (1963). He completed his residency at Duke University and spent four years at the National Institute of Health followed by four years serving as the Luscious Birch Professor and the director of the Reproductive Biology Center at Vanderbilt University. He then moved to Baylor as Professor and Chairman of Molecular and Cellular Biology.

O'Malley's laboratory discovered that steroid hormones and nuclear receptors act on genes to regulate synthesis of messenger RNAs. He then went on to discover the 'missing link coregulators' (coactivators/corepressors) that decipher all of the transcriptional instructions in the receptors. Coactivators are 'master genes' that have immense regulatory influences on tissue development and physiology because they activate subfamilies of genes in a manner designed to coordinately regulate cell physiology and metabolism and growth. Of course, dysfunctions in coactivators (or corepressors) lead to serious genetic, reproductive, metabolic, or oncogenic disease consequences but can serve new markers for diagnosis and therapeutic targets.

He has published over 600 papers and holds 23 patents in the fields of gene regulation, molecular endocrinology and steroid receptors and transcriptional coactivators. Dr O'Malley is considered the father of Molecular Endocrinology and is a member of the National Academy of Sciences and the Institute of Medicine. He has received over 60 honors and awards for his scientific achievements. He was awarded the National Medal of Science by U.S. President George W Bush in 2008.

SfE Transatlantic Medal Lecture

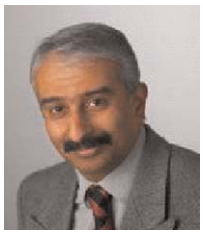
Mitchell A Lazar, University of Pennsylvania, Philadelphia, USA

Dr Mitchell Lazar is the Sylvan Eisman Professor of Medicine and Genetics, the Chief of the Division of Endocrinology, Diabetes, and Metabolism, and the Director of the Institute for Diabetes, Obesity, and Metabolism at the University of Pennsylvania. He received his undergraduate degree in Chemistry from the Massachusetts Institute of Technology, then received a PhD in Neurosciences and an MD from Stanford University. He trained in Internal Medicine at Brigham and Women's Hospital and in Endocrinology at the Massachusetts General Hospital before joining the University of Pennsylvania faculty in 1989.

Dr Lazar's identification of the nuclear heme receptor Rev-erbα and its corepressor complex, and his pioneering studies of PPARγ incluing discovery of the adipocyte hormone resistin, have linked basic mechanisms of gene transcription to physiology and metabolic diseases. He has given named lectures throughout the world, and has served as a member of the Board of Scientific Councilors of the NIDDK as well as many editorial and scientific advisory boards. He has been elected to the American Society for Clinical Investigation and the Association of American Physicians, and received two NIH Merit Awards, the Van Meter Award of the American Thyroid Association, the BMS Freedom to Discover Award, the Richard Weitzman Award, Edwin B. Astwood Lecture Award and Gerald D. Aurbach Lecture Award from The Endocrine Society, and the Stanley Korsmeyer Award of the American Society for Clinical Investigation. He was elected to the Institute of Medicine of the National Academy of Sciences in 2006, and to the American Academy of Arts and Sciences in 2008.

British Thyroid Association Pitt-Rivers Lecture

Professor K Chatterjee, University of Cambridge, Cambridge, UK



Krishna Chatterjee is Professor of Endocrinology at the University of Cambridge, and a Wellcome Trust Senior Investigator. His research interests in genetic and molecular endocrinology include Disorders of Thyroid Hormone Action. He co-directs a national referral laboratory for unusual and discordant thyroid function tests in Cambridge.

Clinical Endocrinology Trust Lecture

Professor David Ray, University of Manchester, Manchester, UK

David Ray graduated in Medicine from University of Manchester in 1987. He trained in medicine and endocrinology in the North West, before doing a PhD with Anne White and Julian Davis, again in Manchester. Towards the end of his PhD he was offered a post with Shlomo Melmed at UCLA, and, attracted by the lab, and the adjacent coast, headed west. This research post stimulated an interest in inflammation, and its neuroendocrine control, which he pursued back in the UK initially as a lecturer, research fellow, and then Professor of Medicine at the University of Manchester. He now runs a research group looking at nuclear receptors, circadian clocks, and inflammation. In addition to research he contributes to the tertiary endocrine service at Manchester Royal Infirmary, and is associate dean for research at the University of Manchester.

SfE Hoffenberg International Medal Lecture

Robert McLachlan, Prince Henry's Institute of Medical Research, Melbourne, Australia

Professor Robert McLachlan, FRACP, PhD is a Principal Research Fellow of the Australian NH&MRC and Director of Clinical Research at Prince Henry's Institute of Medical Research in Melbourne, Australia. His interests include male reproductive endocrinology and the evaluation and management of male infertility, and androgen physiology. He is Deputy Director of Endocrinology, Monash Medical Centre and Consultant Andrologist to the Monash IVF program with research interests in the genetics of male infertility. He has published more than 190 original papers and reviews and is Editor of the Male Reproduction Section of www.ENDOTEXT.org. He is a past President of the Fertility Society of Australia and currently a Consultant to the World Health Organisation on male fertility regulation. He is Director of the Andrology Australia, a Federal Government initiative committed to community and professional education and research in male reproductive health.

Plenary Lectures

SfE Dale Medal Lecture 2013
PL1

http://www.endocrine-abstracts.org/ea/0031/ea0031pl1.htm

DOI: 10.1530/endoabs.31.PL1

SfE Dale Medal Lecture 2014
PL2

Nuclear receptor coactivators: master genes for physiology and pathology

Bert W O'Malley
Baylor College of Medicine, Houston, Texas, USA.

Nuclear receptors control gene expression by recruiting transcriptional coactivators (or corepressors). The coactivators are 'master regulators' that coordinately activate multiple distinct transcription factors and target genes and pathways to control major physiologic processes such as reproduction, development, inflammation, metabolism and growth. Because of their central role as regulatory 'nodes', coactivators are major targets in the development of numerous inherited and acquired endocrine-related pathologies such as infertility, endometriosis, disorders of carbohydrate, lipid and protein metabolism, and especially, numerous cancers. Metabolism and growth are especially prominent pathways for coordinate regulation by coactivators such as SRC-2 and SRC-3. The pleiotropic functions of coactivators in pathways are the result of combinatorial posttranslational modifications of the proteins via enzyme cascades, in conjunction with certain biological isoforms of the proteins. In metabolic diseases and cancers, the intracellular concentrations and the directed 'activities' of the coactivator proteins via posttranslational modifications are critical for 'driving' the transcription-dependent physiological outcomes. However, in the case of the cancer cell's motility or in endometriosis, it is the coactivator protein's isoforms that are adjunct mediators of the disease progression. Thus, as a class, the coactivator proteins provide important insights to polygenic diseases. They also may represent new 'first-in-class' types of potential targets for therapeutic intervention.

DOI: 10.1530/endoabs.34.PL2

Clinical Endocrinology Trust Visiting Professor Lecture
PL3

Adrenocortical tumours: from bench to bedside and back

Bruno Allolio
University Hospital Wuerzburg, Wuerzburg, Germany.

Understanding of the molecular pathogenesis of adrenocortical neoplasias has been greatly advanced by exome sequencing demonstrating in Conn adenomas hot spot mutations in KCJN5, ATP1A1, ATP2B in a substantial subgroup of patients. Current work now demonstrates that constitutive activation of the cAMP – PKA pathway not only causes rare bilateral hyperplasias like PPNAD, but is also involved in a high percentage of cortisol producing adenomas. Intriguingly, this hot spot mutation is only seen in overt Cushing's syndrome (CS), but not in subclinical CS or cortisol secreting adrenocortical cancer (ACC). Deep sequencing of ACC is currently performed by an international consortium and results are expected by the end of 2013. It is hoped that this analysis will reveal new targets for therapy, as current treatment options for advanced ACC remain unsatisfactory. The role of surgery for recurrent ACC has now been clarified by recent large retrospective studies indicating that surgery is most helpful in late recurrence and when complete resection of metastatic disease seems feasible. Mitotane remains the most important drug for ACC. However, due its strong induction of Cyp3A4 it impairs the activity of many new targeted therapies (e.g. sunitinib) making it a double edged sword. Current work, therefore, tries to better clarify the mechanism of action of mitotane with the goal to select useful drugs for combination therapy and to identify responders prior treatment.

Generously supported by *Clinical Endocrinology Trust*.
DOI: 10.1530/endoabs.34.PL3

SfE European Medal Lecture
PL4

Mechanisms of thyroid hormone resistance

Theo Visser
Erasmus University Medical Center, Rotterdam, The Netherlands.

Thyroid hormone (TH) is essential for the development of tissues, in particular the brain, and for their metabolic function throughout life. The thyroid secretes mostly the prohormone T_4 and a small amount of the active hormone T_3. Most T_3 is generated by outer ring deiodination of T_4 in peripheral tissues, and both T_4 and T_3 are degraded by inner ring deiodination to inactive metabolites. This involves three different deiodinases (D1–3).

Most actions of TH are initiated by binding of T_3 to nuclear receptors (TRs), which results in a change in the expression of TH responsive genes. TRs are encoded by two genes; THRA is located on human chr 17 and THRB on chr 3. Both genes give rise to different proteins of which TRα1, TRβ1 and TRβ2 represent bonafide T_3-binding receptors.

The active sites of the deiodinases and the receptors are located inside the cell. Both metabolism and action of TH therefore require the transport of the hormone across the plasma membrane mediated by specific TH transporters. Multiple transporters have been identified but only three of them are specific for TH, namely MCT8, MCT10 and OATP1C1.

The syndrome of thyroid hormone resistance β (RTHβ) has been known for a long time. RTHβ is characterized by a combination of elevated FT_4 and non-suppressed TSH levels. The clinical phenotype is usually mild but may comprise among others tachycardia and atrial fibrillation. RTH is caused by a heterozygous mutation in the T_3-binding domain of TRβ1/2, resulting in an impaired negative feedback of TH on TSH secretion mediated by TRβ2.

Only very recently patients have been identified with heterozygous mutations in TRα1 resulting in RTHα. These patients show a strongly delayed bone development and mildly delayed mental and motor development. This is explained by the important expression of TRα in bone and brain. These patients have low FT_4 and high T_3 but normal TSH levels.

TH resistance may also be caused by an impaired cellular transport of the hormone. This has been demonstrated in patients with mutations in MCT8, which gene is located on the X chromosome. Males with hemizygous mutations in MCT8 suffer from severe psychomotor retardation. They also have low FT_4 and high T_3 but usually normal TSH levels.

DOI: 10.1530/endoabs.34.PL4

SfE Transatlantic Medal Lecture
PL5

Transcriptional coordination of circadian and metabolic physiology

Mitchell Lazar
University of Pennsylvania, Philadelphia, Pennsylvania, USA.

Organismal metabolic homeostasis is normally accomplished by the physiological functions of several metabolic tissues, including liver, muscle, and fat. The endocrine system coordinates organismal metabolic homeostasis via hormones, such as insulin and glucocorticoids, that act on metabolic tissues to partition nutrients within a physiological range. Metabolic diseases, such as obesity

and diabetes, ensue when these homeostatic systems fail. Normal metabolic physiology also displays circadian rhythmicity that likely evolved to anticipate recurrent, daily shifts between periods of inactivity/fasting and activity/feeding. The nuclear heme receptors Rev-erbα and β are potent transcription repressors whose expression in peripheral tissues is highly circadian. The Rev-erbs function as redundant components of the molecular clock as well as direct regulators of circadian gene expression. In liver, Rev-erbs rhythmically recruit the NCoR-HDAC3 corepressor complex to target genes across the genome, generating an oscillating epigenome and controlling circadian hepatic lipid metabolism. In brown adipose tissue, Rev-erbα expression is antiphase to the body temperature rhythm, and its repression of uncoupling protein 1 contributes to the daily nadir of body temperature as well as to a physiological rhythm of tolerance to cold ambient temperature. The role of oscillating Rev-erb expression in the integrated physiology of circadian rhythms and metabolism will be discussed.

DOI: 10.1530/endoabs.34.PL5

BTA Pitt-Rivers lecture

PL6

Disorders of thyroid hormone action: insights from human genetics

Krishna Chatterjee
University of Cambridge, Cambridge, UK.

Disorders of thyroid hormone action are classified broadly, to encompass conditions with defective cellular uptake, metabolism or nuclear action of thyroid hormones. Mutations in SECISBP2 cause a multisystem disorder of defective selenoprotein synthesis, with features due to tissue-specific selenoprotein deficiencies (e.g. male infertility, muscular dystrophy), raised cellular reactive oxygen species due to lack of antioxidant selenoenzymes (e.g. photosensitivity, increased adipose mass and function), associated with a biochemical signature due to impaired conversion of T_4 to T_3 via selenium-containing deiodinases. Genomic thyroid hormone action is mediated via receptor subtypes (TRα, TRβ) with differing tissue distributions. TRβ-mediated resistance to thyroid hormone (RTH) is characterised by elevated thyroid hormones, raised metabolic rate and cardiac hyperthyroidism but hepatic resistance (dyslipidaemia, steatosis). In contrast, TRα1-mediated RTH patients exhibit growth retardation, skeletal dysplasia and constipation together with reduced metabolic rate and cardiac hypothyroidism, with near-normal thyroid hormone levels. The contrasting phenotypes of TRα1 and β- mediated RTH exemplify the differing importance of receptor subtypes in tissues, providing a rational basis for receptor-specific drug development.

Partially supported by *Clinical Endocrinology Trust*.

DOI: 10.1530/endoabs.34.PL6

SfE Medal Lecture

PL7

The clock in the pituitary gland: timing annual cycles

Andrew Loudon
University of Manchester, Manchester, UK.

Biological clocks drive the physiology and behaviour of all organisms, from bacteria to humans. Much attention has focused on the circadian 24 h clock, and rapid progress made in defining key molecular components that regulate our rhythmic physiology. The circadian clock also drives a linked timing system that controls annual cycles of hormone secretion and metabolism, this is most evident in wild species, such as hibernating mammals. Annual cycles are also key feature of domesticated animals, where seasonal clocks drive reproduction, growth and behaviour. Remarkably, a hitherto under-investigated pituitary structure – the pars tuberalis (PT) – appears to be central to the generation of such annual cycles. The PT can measure day length, encoded by nocturnal secretion of melatonin hormone, and TSH of PT origin drives an adjacent circuit regulating thyroid hormone metabolism in the hypothalamus. Thyroid hormone (tri-iodothyronine) activation in the brain leads to altered neuroendocrine activity and rhythmic control of annual reproductive hormones.

In this talk, I will summarise the current state-of-the art investigations of this exciting new timing circuit. I will show that an ancient photoreceptor gene has been co-opted to drive summer-like responses in this tissue, leading to dramatic re-modeling of cells in this endocrine tissue. Re-modeling of epigenetic circuits is likely in this system and I will discuss how clock-controlled methylation/demethylation may lead to long-term changes in neuroendocrine function and annual cycles.

What of humans? Very little is known of the underlying causal mechanisms driving pituitary pathology and endocrine dysfunction. I propose that we may gain important insight into human disease by studying how long-term rhythm generation – driven by clocks – may underpin normal physiology and speculate that perhaps dysregulation of these circuits may also lead to pathology in humans.

DOI: 10.1530/endoabs.34.PL7

Clinical Endocrinology Trust Lecture

PL8

Understanding glucocorticoid action, and the role of the glucocorticoid receptor

David Ray
University of Manchester, Manchester, UK.

The glucocorticoid receptor (GR) is a ligand activated transcription factor, serving to regulate both energy metabolic and immune functions. The natural endogenous glucocorticoid in humans is cortisol, but a variety of synthetic molecules have been developed to treat inflammatory disease. These synthetic ligands offer new insights into how the GR works, and how it can be manipulated. Novel, non-steroidal GR agonists specifically alter the GR LBD structure at the HSP90 binding site. Despite their high affinity the non-steroidal ligands induce surprisingly slow GR nuclear translocation, followed by prolonged duration of action. These non-steroidal ligands predict new anti-inflammatory drugs with prolonged duration of action due to altered pharmacodynamics rather than altered pharmacokinetics.

Downstream of ligand activation the cellular response to GR varies greatly by time of day, between tissues and within populations. We adopted a systems biology approach to understand the basis for variation in glucocorticoid response. In this way we stratified a healthy human cohort by glucocorticoid response, and profiled the genes expressed in isolated T lymphoblasts, thereby discovering genes whose expression associated with *in-vivo* glucocorticoid sensitivity. This approach revealed two proteins, BMPRII, and IFI16, as novel regulators of the glucocorticoid signalling cascade.

In further studies we employed proteomics to identify novel proteins bound to the glucocorticoid receptor. These studies identified a different spectrum of protein partners under basal, and glucocorticoid treated conditions, with a shift from cytoplasmic and membrane partners under the former, and nuclear and chromatin components in the latter. We used these insights to determine how the membrane protein caveolin binds to, and regulates the glucocorticoid receptor, and how this regulation may play a role in the regulation of inflammation.

Taken together we have discovered new controls regulating glucocorticoid action in cells, and also found new mechanisms explaining glucocorticoid regulation of inflammation.

Generously supported by *Clinical Endocrinology Trust*.

DOI: 10.1530/endoabs.34.PL8

SfE Hoffenberg International Medal Lecture

PL9

Male fertility regulation: achievements and frustrations

Robert McLachlan[1,2,3]
[1]Prince Henry's Institute of Medical Research, Clayton, Victoria, Australia; [2]Department of Obstetrics and Gynaecology, Monash University, Clayton, Victoria, Australia; [3]Monash IVF Pty Ltd, Richmond, Victoria, Australia.

Men actively participate in family planning and fertility treatments. Much is now understood about the hormonal regulation of spermatogenesis and its therapeutic manipulation. Hypogonadotropic hypogonadism is uncommon but provides the opportunity to restore natural fertility using gonadotrophin therapy. Prenatal-onset (e.g. Kallmann's), cryptorchidism and small testes predict a poorer spermatogenic response compared to post pubertal onset cases; natural fertility occurs in $\sim$70 and $>$90%, respectively. Most infertility arises from poor spermatogenesis; a defined cause is apparent in the minority (Klinefelters, Yq microdeletions, testicular damage) with most cases being unexplained. Genetic causations are emerging from translational studies using clinical DNA databases. The management of idiopathic male infertility has been transformed by the development of intracytoplasmic sperm injection. When viable sperm are retrievable from the semen or the genital tract, success rates in men previously considered sterile are similar to those of age-matched couples with other aetiologies.

On the other hand a demand exists for new safe, effective and reversible male contraceptive options. Male hormonal contraception relies on gonadotrophin suppression by exogenous androgens, often combined with progestin, while maintaining virilisation. Spermatogenesis is impaired by loss of FSH action and a 100-fold reduction in intratesticular testosterone levels. Over 3–6 months sperm densities fall to $<$1 million/ml with contraceptive efficacy similar to female methods. Inadequate suppression in $\sim$5% of men probably results from residual intratesticular androgen action. Despite decades of encouraging translational studies, including a recent multinational trial sponsored by the WHO and CONRAD, development has stalled with the withdrawal of industry support due to uncertainty about the risk:benefit ratio (registration costs, product liability, side effect profile and market size). It is unrealistic to expect 100% applicability, acceptability and efficacy without side effects. Public sector supported research continues especially the use of selective androgen response modulators and the identification of non-hormonal approaches.

DOI: 10.1530/endoabs.34.PL9

Society For Endocinology Journal Awards

Society for Endocrinology Journal Award – *Journal of Endocrinology*

JA1

Mice deficient in PAPP-A show resistance to the development of diabetic nephropathy

Jessica R Mader[1], Zachary T Resch[1], Gary R McLean[3], Jakob H Mikkelsen[4], Claus Oxvig[4], Ronald J Marler[2] & Cheryl A Conover[1]
[1]Endocrine Research Unit, Division of Endocrinology, Mayo Clinic, 200 First Street SW, 5-194 Joseph, Rochester, Minnesota 55905, USA; [2]Department of Comparative Medicine, Mayo Clinic, Collaborative Research Building, 13400 E Shea Boulevard, Scottsdale, Arizona 85259, USA; [3]Cellular and Molecular Immunology Research Centre, London Metropolitan University, London, UK; [4]Department of Molecular Biology and Genetics, Aarhus University, Gustav Wieds Vej 10C, DK-8000 Aarhus C, Denmark.

Journal of Endocrinology 2013 **219** 51–58. DOI: 10.1530/JOE-13-0167

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

JA2

Thyroid-specific inactivation of KIF3A alters the TSH signaling pathway and leads to hypothyroidism

Eva D'Amico[1], Stéphanie Gayral[1], Claude Massart[2], Jacqueline Van Sande[2], Jeremy F Reiter[3], Jacques E Dumont[2], Bernard Robaye[1] & Stéphane Schurmans[1,4,5,6]
[1]Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM), Institut de Biologie et de Médecine Moléculaires (IBMM), Université Libre de Bruxelles (ULB), Rue des Professeurs Jeener et Brachet 12, 6041 Gosselies, Belgium; [2]IRIBHM, Université Libre de Bruxelles (ULB), Campus Erasme, Route de Lennik 808, 1070 Brussels, Belgium; [3]Department of Biochemistry and Biophysics, University of California, Smith Cardiovascular Research Building, Mission Bay Boulevard South 555, San Francisco, California 94158-9001, USA; [4]Laboratoire de Génétique Fonctionnelle, GIGA-Research Centre, Université de Liège (ULg), Rue de l'Hôpital 1, 4000 Liège, Belgium; [5]Welbio, Université de Liège, Liège, Belgium; [6]Département des Sciences Fonctionnelles, Secteur de Biochimie Métabolique, Université de Liège, Boulevard de Colonster 20, 4000 Liège, Belgium.

Journal of Molecular Endocrinology 2013 **50** 375–387. DOI: 10.1530/JME-12-0219

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

JA3

Highly prevalent TERT promoter mutations in aggressive thyroid cancers

Xiaoli Liu[1], Justin Bishop[2], Yuan Shan[3], Sara Pai[4], Dingxie Liu[1], Avaniyapuram Kannan Murugan[1], Hui Sun[5], Adel K El-Naggar[6] & Mingzhao Xing[1]
[1]Laboratory for Cellular and Molecular Thyroid Research, Division of Endocrinology and Metabolism, Johns Hopkins University School of Medicine, 1830 East Monument Street, Suite 333, Baltimore, Maryland 21287, USA; [2]Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287, USA; [3]Departments of Anatomic Pathology and Neuro-Oncology, Moffitt Cancer Center, Tampa, Florida 33612, USA; [4]Department of Otolaryngology–Head and Neck Surgery, Johns Hopkins University School of Medicine, Baltimore, Maryland 21287, USA; [5]Jilin Provincial Key Laboratory of Surgical Translational Medicine, Department of Thyroid and Parathyroid Surgery, China-Japan Union Hospital, Jilin University, Changchun, Jilin Province 130033, China; [6]Department of Pathology, University of Texas MD Anderson Cancer Center, Houston, Texas 77030, USA.

Endocrine-Related Cancer 2013 **20** 603–610. DOI: 10.1530/ERC-13-0210

Society for Endocrinology Journal Award – *Clinical Endocrinology*

JA4

What predicts adverse outcomes in untreated primary hyperparathyroidism? The Parathyroid Epidemiology and Audit Research Study (PEARS)

Ning Yu[1,3], Graham P Leese[2,3] & Peter T Donnan[1]
[1]Dundee Epidemiology and Biostatistics Unit, Division of Population Health Sciences, Dundee, UK; [2]Ninewells Hospital and Medical School, University of Dundee, Dundee, UK; [3]Medical Research Institute, University of Dundee, Dundee, UK.

Clinical Endocrinology 2013 **79** 27–34. DOI: 10.1111/cen.12206

Society for Endocrinology Journal Award – *Endocrine Connections*

JA5

PKA regulatory subunit R2B is required for murine and human adipocyte differentiation

Erika Peverelli[1], Federica Ermetici[1,2], Sabrina Corbetta[3], Ettore Gozzini[1], Laura Avagliano[4], Marco A Zappa[5], Gaetano Bulfamante[4], Paolo Beck-Peccoz[1], Anna Spada[1] & Giovanna Mantovani[1]
[1]Endocrinology and Diabetology Unit, Department of Clinical Sciences and Community Health, University of Milan, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Pad.Granelli, Via F. Sforza, 35, 20122 Milan, Italy; [2]Diabetology and Metabolic Disease Unit, IRCCS Policlinico San Donato, San Donato Milanese, Italy; [3]Endocrinology Unit, IRCCS Policlinico S.Donato, Department of Biomedical Sciences for Health, University of Milan, Milan, Italy; [4]Unit of Human Pathology, Department of Health Sciences, San Paolo Hospital Medical School, University of Milan, Milano, Italy; [5]Surgical Department, Ospedale Sacra Famiglia Fatebenefratelli, Erba, Italy.

Endocrine Connections 2013 **2** 196–207. DOI: 10.1530/EC-13-0049

Symposia

GNRH biology – from pulses to longevity (Supported by *Journal of Molecular Endocrinology*)

S1.1

Systems approaches to understanding GnRH signalling

Rebecca Perrett[1], Margaritis Voliotis[2], Stephen Armstrong[1], George Pope[1], Krasimira Tsaneva-Atanasova[1,3] & Craig McArdle[1]
[1]School of Clinical Sciences, University of Bristol, Bristol, UK; [2]School of Mathematics, University of Bristol, Bristol, UK; [3]Department of Mathematics, University of Exeter, Exeter, UK.

GnRH acts via Gq/11-coupled GPCRs on gonadotropes to control synthesis and secretion of LH and FSH. We use mathematical and statistical approaches as well as automated cell imaging to explore this system. In cells expressing ERK2–GFP and GnRH receptors, GnRH pulses cause rapid and transient nuclear translocation of ERK2–GFP, providing a live-cell readout for ERK activation[1]. A mathematical model trained against this data predicted greater sensitivity to receptor number than to GnRH concentration. It also predicted greater sensitivity to pulse frequency than to pulse width because of activation continuing beyond the GnRH pulse. Each of these predictions was confirmed experimentally. Single-cell ERK measures can also be used to quantify the efficiency of information transfer. Specifically, mutual information (MI) measures accuracy of information processing, as the maximal number of inputs a cell can resolve without error[2]. To measure MI we expressed Egr1-zsGREEN (an imaging readout for ERK-driven transcription) in LβT2 cells and also stained for ppERK1/2, constructing concentration–response curves at varied times. Imaging data from ∼100 000 individual cells and imposing a uniform input distribution over the GnRH concentration range revealed ppERK1/2 MI values increasing rapidly to ∼0.8 bits at 5 min, then reducing to ∼0.6 bits at 240 min. MI for Egr1-zsGREEN increased from <0.1 to ∼ 0.8 bits between 60 and 240 min, revealing the time-dependent transfer of information from ERK1/2 to the transcriptional readout. MI of 1 bit has been interpreted as meaning that individual cells can distinguish only two states of the environment[2]. Our data therefore indicate inefficient information processing, in spite of the fact that GnRH effects are graded over a broad concentration range. This could reflect the importance of additional information from other branches of the signalling network.

References
1. Armstrong SP *et al. J Biol Chem* 2010 **285** 24360.
2. Cheong R *et al. Science* 2011 **334** 354.

DOI: 10.1530/endoabs.34.S1.1

S1.2

Abstract unavailable.

DOI: 10.1530/endoabs.34.S1.2

S1.3

GnRH and inflammatory signalling in the ageing hypothalamus

Dongsheng Cai
Albert Einstein College of Medicine, Bronx, New York, USA.

In this talk, I will discuss the integrated role of hypothalamic inflammation and GnRH dysfunction in the development of systemic aging. First, I will provide an overview on aging-related profiles of hypothalamic pro-inflammatory NF-kB pathway as well as GnRH change. Second, I will describe how these changes can employ a neurodegenerative mechanism, in particular through targeting hypothalamic neural stem cells, to mediate the pathogenesis of aging and many aging-related disorders. Finally, I will discuss the negative impact of hypothalamic NF-kB pathway on the level and function of GnRH, which underlies the induction of aging-related neurogenic impairment, and further provide evidence showing that inhibition of hypothalamic inflammation or restoration of GnRH function can be useful for slowing down aging and related whole-body declines. Altogether, hypothalamic inflammation and related neuroendocrine dysfunction may work as a central process for the development of systemic aging and therefore various aging-related diseases.

DOI: 10.1530/endoabs.34.S1.3

The upside of glucocorticoids in metabolism (Supported by *Journal of Endocrinology*)

S2.1

Insulin sensitization of adipose tissue by glucocorticoids

Jeremy Tomlinson
University of Birmingham, Birmingham, UK.

Current dogma suggests that glucocorticoids (GCs) cause insulin resistance in all tissues. Whilst it is clear that they cause global, whole body insulin resistance, we have challenged the concept that the actions of GCs are the same in all tissues. Using a variety of human cell-based models, we have shown that in contrast to their actions in skeletal muscle and liver, GCs cause insulin sensitization in human adipose tissue, enhancing insulin-stimulated PKB/akt phosphorylation, glucose uptake, and lipogenesis. Whilst these effects persist for up to 7 days, there are, in addition, depot specific differences; our observations seem confined to the subcutaneous adipose tissue depot. In omental cells, GCs cause neither insulin sensitization, nor insulin resistance. Whilst it is possible that our observations reflect *in vitro* culture systems, we have recently extended our findings to a translational randomized, double blind, placebo controlled, cross-over study. Patients were treated with either overnight hydrocortisone or saline infusions in a random order. Following GC treatment, we observed hepatic and skeletal muscle insulin resistance, however, insulin-mediated suppression of adipose tissue lipolysis was enhanced, as was insulin-stimulated pyruvate generation. These data that are consistent with adipose tissue insulin sensitization.
Our studies have demonstrated opposing metabolic actions of GCs that challenge current textbook definitions and highlight the importance of tissue-specific assessments in clinical studies.

DOI: 10.1530/endoabs.34.S2.1

S2.2

Maintaining the anti-inflammatory action of GC without the metabolic side effects

Carolyn Cummins[1,2], Rucha Patel[1], Ricky Tsai[1] & Arturo Orellana[3]
[1]University of Toronto, Toronto, Ontario, Canada; [2]Banting and Best Diabetes Centre, Toronto, Ontario, Canada; [3]York University, Toronto, Ontario, Canada.

Synthetic glucocorticoids (GCs) including dexamethasone (Dex) are potent anti-inflammatory drugs. However, long-term use of GCs results in deleterious side effects such as hyperglycemia, hepatosteatosis, and insulin resistance. The nuclear hormone receptors LXRα and LXRβ are known for their important roles in modulating whole-body cholesterol homeostasis. Recently, we have shown that liver X receptor β (LXRβ) knockout mice are protected against Dex-induced hyperglycemia and hepatosteatosis, without affecting Dex-mediated inflammatory suppression (Patel *et al. J Clin Invest*, 2011). From our studies, we proposed a model whereby upon GR activation, hepatic LXRβ is required to achieve maximal induction of the gluconeogenic pathway in the liver but is not required for GR-mediated suppression of the immune system. To determine whether the interplay between LXRβ and GR could be exploited for future therapeutic intervention, we tested whether LXRβ antagonists would mimic the LXRβ−/− phenotype in this context. GSK2033, a potent pan-LXR antagonist, was dosed to LXRα−/− primary hepatocytes and/or mice alone or in combination with Dex. Herein, we show that antagonizing LXRβ activity (by GSK2033) along with Dex co-administration reduced Dex-dependent Pepck induction and glucose production in primary hepatocytes. Moreover, *in situ* perfusion of GSK2033 and Dex via the portal vein decreased GR and Pol II recruitment to the Pepck promoter in LXRα−/− livers. In addition, Dex-mediated inflammatory suppression remained unaffected by GSK2033 co-treatment in mouse primary macrophages stimulated with LPS. These findings demonstrate that the undesired metabolic effects of therapeutic GCs might be avoided by concurrent pharmacologic antagonism of hepatic LXRβ; without affecting the desired anti-inflammatory effects of GCs.

DOI: 10.1530/endoabs.34.S2.2

S2.3

A hair 'o' the dog that bit you? Elevated pancreatic β cell glucocorticoid reactivation protects against β cell failure

Nik Morton
University of Edinburgh, Edinburgh, UK.

Excessive glucocorticoid action is detrimental to metabolic health. The last 15 years has seen the emergence of enzymatic intra-cellular glucocorticoid reactivation, driven by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), as a key mechanism contributing to glucocorticoid action. In most contexts, high intracellular glucocorticoid regeneration results in adverse metabolic effects. Inhibition or gene knockout of 11β-HSD1 is metabolically protective. Transgenic overexpression of 11β-HSD1 in adipose tissue and liver produces local and systemic defects in insulin sensitivity and nutrient metabolism akin to those found in human 'metabolic syndrome'. Elevated 11β-HSD1 was also found in pancreatic islets from rodent 'diabesity' models. 11β-HSD1 activity was hypothesized to suppress insulin secretion and promote diabetes. We tested this hypothesis by overexpressing 11β-HSD1 specifically in β-cells using the mouse-I insulin promoter. Unexpectedly, we found that modest 11β-HSD1 overexpression protected β-cells against chronic high fat-diet-mediated dysfunction. Protection was associated with induction of a 'super-differentiated' state and reduced cellular stress that augmented insulin secretion. More recently we have found that mice overexpressing 11β-HSD1 in β-cells are protected from damage caused by the β-cell toxin streptozotocin. This effect is associated with modulation of inflammation and inflammatory resolution processes linked to β-cell destruction and survival. The unexpected beneficial effects of β-cell 11β-HSD1 elevation in these distinct contexts has prompted us to re-evaluate some potential therapeutic applications of glucocorticoid precursors.

DOI: 10.1530/endoabs.34.S2.3

Emerging Clinical importance of iodine (Supported by *Endocrine-Related Cancer*)

S3.1

Inadequate iodine status in UK pregnant women adversely affects cognitive outcomes in their children: results from the Avon Longitudinal Study of Parents and Children (ALSPAC)

Margaret Rayman[1], Sarah Bath[1], Colin Steer[2], Jean Golding[2] & Pauline Emmett[2]
[1]University of Surrey, Guildford, UK; [2]University of Bristol, Bristol, UK.

As a component of thyroid hormones, iodine is essential for the development of the fetus, particularly for brain development. Though there has been no awareness of a problem of iodine deficiency in the UK for many years, it is becoming increasingly apparent that mild-to-moderate iodine-deficient is now present in certain population sub-groups. We assessed whether such a level of deficiency in UK pregnant women could be having an adverse effect on cognition in their offspring using samples and data from the Avon Longitudinal Study of Parents and Children (ALSPAC). Urinary iodine concentration (and creatinine to correct for urine dilution) was measured in samples from 1040 first-trimester pregnant women that had been stored at −20 °C. Based on the WHO criteria for deficiency/sufficiency in pregnancy, women's results were dichotomised to <150 or ≥ 150 μg iodine/g creatinine. The relationships between maternal iodine status and child's IQ at age 8 and reading ability at age 9 were analysed. Socio-economic, parental and child factors ($n=21$) were included as confounders. Overall the group was found to be mildly-to-moderately iodine deficient based on a median urinary iodine concentration of 91.1 μg/l (110 μg/g creatinine). Children of women with an iodine:creatinine ratio <150 μg/g were more likely to have scores in the lowest quartile for verbal IQ (OR 1.58, 95% CI 1.09–2.30), reading accuracy (OR 1.69, 95% CI 1.15–2.49), and reading comprehension (OR 1.54, 95% CI 1.06–2.23) than were children of mothers with an iodine:creatinine ratio ≥ 150 μg/g. When the <150 μg/g group was sub-divided, scores worsened on going from ≥ 150 to 50–150 μg/g to <50 μg/g. Our results show the importance of adequate iodine status during early gestation and highlight the risk that even a mild-to-moderate level of iodine deficiency can pose to the developing infant. This situation needs to be addressed urgently through public-health policy.

DOI: 10.1530/endoabs.34.S3.1

S3.2

Regulation of NIS function in thyroid and breast cancer

Gregory Brent
David Geffen School of Medicine at UCLA, Los Angeles, California, USA.

Expression of the sodium iodide symporter (NIS) protein, and correct membrane insertion, is required for efficient iodide uptake in thyroid and lactating breast. Radioactive iodide is routinely utilized to target remnant thyroid and metastasis after total thyroidectomy for thyroid cancer. Stimulation of NIS expression by high levels of TSH, however, is necessary to achieve radioiodide uptake into thyroid cancer sufficient for therapy. The majority of breast cancer also expresses NIS protein, but iodide uptake is low and insufficient for routine radioiodine therapy. Retinoic acid is a potent NIS inducer in some breast cancer, but generally not in thyroid cancer. NIS expression and membrane insertion is regulated by activation of signal transduction pathways that are distinct in thyroid and breast cancer. Spontaneous mutations in kinases leads to stimulation of some of these pathways in cancer. Inhibitors have been used to treat aggressive cancer and, in some cases, these inhibitors induce redifferentiation of the cells and restore iodide uptake. Inhibition of NIS membrane insertion by the pituitary tumor-transforming gene 1 protein-binding factor (PBF) may also play a role in regulating iodide uptake in both thyroid and breast cancer. Differential regulation of NIS in thyroid and breast cancer provides potential targets for more efficient radioiodide therapy in thyroid cancer and new approaches to breast cancer therapy. These approaches may also be relevant to systemic radioiodide treatment of a range of other cancers, which do not express endogenous NIS, but can be treated with tumor-selective introduction of exogenous NIS.

DOI: 10.1530/endoabs.34.S3.2

S3.3

Use of radioiodine in the imaging and therapy of non-thyroidal tumors

Christine Spitzweg
University Hospital Munich, Ludwig-Maximilians-University Munich, Munich, Germany.

As one of the oldest targets for molecular imaging and targeted radionuclide therapy, characterization of the sodium iodide symporter (NIS) as a novel reporter and suicide gene offers the possibility of NIS gene transfer in nonthyroidal tumors followed by diagnostic and therapeutic application of radioiodine. Our previous work has convincingly demonstrated the high efficacy of radionuclide therapy after tumor-selective NIS gene delivery. As logical consequence of our pioneer studies, the next crucial step towards clinical application of the promising NIS gene therapy concept is the evaluation of gene transfer methods that own the potential to achieve sufficient tumor-selective transgene expression levels after systemic application to be able to reach tumor metastases. For this purpose, the potential of novel polyplexes alone and for surface modification of replication-selective adenoviral vectors have been evaluated for systemic NIS gene delivery in addition to mesenchymal stem cells as gene delivery vehicles. Based on its dual function as reporter and therapy gene, NIS has been used in our studies for non-invasive imaging of vector biodistribution followed by monitoring of the therapy response after NIS-targeted radionuclide therapy.

DOI: 10.1530/endoabs.34.S3.3

Putting flesh on the bones

S4.1

Assessment of abnormalities of skeletal development

Esther Kinning
West of Scotland Genetics Service, Southern General Hospital, Glasgow, UK.

The skeletal dysplasias are a heterogeneous group of over 450 conditions characterised by abnormalities in growth, development, or differentiation of the skeleton. They are classified according to the International Nosology and Classification of Genetic Skeletal Disorders into 40 different classes reflecting the broad range of phenotypes which vary in severity from perinatal lethality to mild short stature. Broadly, they can be divided into conditions affecting bone mineral homeostasis (osteodysplasias), disorders affecting a single bone or group of bones (dysostoses) or conditions resulting from abnormal cartilage development leading to deranged development of bone and growth (chondrodysplasias).

Many will present with abnormal growth in childhood but complications such as joint deformity and early-onset osteoarthritis add to the disease burden in adult patients.
Elucidation of the genetic mechanisms underlying these disorders has allowed us to understand more about their aetiology and directed research into potential therapeutic options. I will present a practical clinical approach to the diagnosis of disorders of skeletal development and will discuss relevant features in the history and examination and appropriate investigation. I will touch upon management of more common conditions and future avenues of therapy.

DOI: 10.1530/endoabs.34.S4.1

S4.2

Osteogenesis imperfecta: management beyond childhood

Neil Gittoes
Queen Elizabeth Hospital, Birmingham, UK.

The natural history of osteogenesis imperfecta (OI) during childhood is changing due to the availability and effectiveness of bisphosphonates in ameliorating fracture risk and promoting significant gains in bone mineral density in most children with OI. Not all children with OI require treatment with bisphosphonates, however. Fracture rates in patients with OI typically fall in the latter teenage years as the longitudinal growth of the skeleton ceases. Most adults with OI enjoy a much reduced risk of fracture (compared to early life) through early adult life but surprisingly little is know of the later natural history of OI under the influence of ageing. The difference in clinical expression of OI through later teenage years necessitates effective transition services from paediatric, often intensively managed patients through to 'lower key' monitoring with a change in emphasis according to patient needs. Management of patients with OI is not solely based on BMD monitoring and assessment of fracture risk but instead should be holistic, integrating the needs of individuals that often relate to hearing loss, dental issues, orthopaedic complications, and disability. Furthermore, there is a need for clear genetic counselling of patients with OI, although routine genetic testing is not advocated unless it is likely to influence management decisions.
Adults with OI do sometimes continue to fracture through adult life and an individualised approach to treatment intervention is required. Conventional anti-fracture drugs are sometimes (but not routinely) employed where fracture risk high. There is no evidence base for such interventions, however. Adult patients with OI should not routinely be treated long-term with bisphosphonates as such patients are at risk of atypical femoral fractures that are associated with prolonged bisphosphonate exposure.

DOI: 10.1530/endoabs.34.S4.2

S4.3

Management of hypophosphatemic rickets

Thomas Carpenter
Yale University, New Haven, Connecticut, USA.

Hypophosphatemia due to excess urinary phosphate losses and rachitic bone disease occur in several related disorders. The most common form of the heritable hypophosphatemic disorders, X-linked hypophosphatemia (XLH, estimated incidence: 1/10 000–1/20 000), is due to loss-of-function mutations of the osteocyte/osteoblast protein, PHEX. Associated elevations in circulating FGF23 lead to reduced abundance of phosphate transporters on the luminal surface of renal tubular cells, and to inappropriately normal (or frankly low) circulating levels of 1,25 dihydroxyvitamin D (1,25D). Thus currently available therapies for XLH employ supplementation with phosphate and 1,25D. The primary clinical manifestations of XLH in childhood are bowing and other leg deformities. Dental disease occurs and is progressively problematic throughout adult life. Other complications in later years include arthritis, calcified entheses, and osteophyte formation, often leading to progressive pain and limited range of motion. FGF23 mediates other forms of hypophosphatemic rickets, including rare autosomal dominant and recessive forms and an acquired variant (tumor induced osteomalacia) which results from paraneoplastic secretion of FGF23 by small, frequently benign tumors. In contrast, hereditary hypophosphatemic rickets with hypercalciuria (HHRH) is due to loss-of-function mutations in the NaPi2c renal phosphate transporter, occurring independently of FGF23 activity. These differing features compel an accurate diagnosis, as treatment strategies differ.
Current replacement with phosphate and 1,25D is a cumbersome approach, is fraught with complications, and does not address all features of the disease. Novel approaches include targeted inhibition of FGF23 action with neutralizing antibodies to FGF23, and with the inactive, receptor binding C-terminus of FGF23; decreasing FGF23 secretion via calcitonin; and stimulation of FGF23 catabolism (via enhancement of subtilisin protein convertase activity). Other strategies aim to reduce secretion of parathyroid hormone, a factor which may enhance FGF23 activity. Treatment for HHRH employs a different strategy altogether, aiming to reduce 1,25D levels and to avoid renal stone risks.

DOI: 10.1530/endoabs.34.S4.3

Endocrine consequences of major trauma

S5.1

The immune-endocrine mechanisms of trauma-induced sarcopenia

Peter Hampson[1], Mark Foster[1,2], Angela Taylor[1], Conor Bentley[2], Joanne Fallowfield[3], Mark Midwinter[1,2], Wiebke Arlt[1] & Janet Lord[1]
[1]University of Birmingham, Birmingham, UK; [2]University Hospital Birmingham, Birmingham, UK; [3]Institute of Naval Medicine, Alverstoke, Hants, UK.

Advances in trauma care have improved survival resulting in more severely injured individuals surviving to enter the trauma care pathway. A significant threat to recovery is now the dysregulated immune response to injury. The hyperinflammatory response of the innate immune system (SIRS), combined with immunoparesis, leads to complications such as multi-organ failure and sepsis. Moreover, recovery from trauma is significantly affected by age, which may be due to changes in the immune and endocrine systems with age that modify the ability to respond appropriately to trauma. Increased understanding of endocrine and immune changes in trauma may influence the development of novel interventions for trauma.
We carried out a prospective cohort study in 102 severely injured patients with an injury severity score (ISS) range 4–75, at the Queen Elizabeth Hospital Birmingham. Blood samples and 24-h urines were collected at baseline (<24 h after major trauma), on days 3, 5, 10, 14, 21, 28 and 2, 3, 4, and 6 months post injury. Serum DHEAS post injury was significantly lower than in healthy controls ($P<0.0001$) and was still lower than controls at 6 months. The cortisol:DHEAS ratio peaked after 20 days and normalised by 6 months. Nitrogen excretion peaked early at 23 gN/day and dropped below 10 gN/day by week 6. Muscle thickness reduced by 31% at 6 weeks and returned to baseline 5 months post injury; this U-shaped curve correlated with the course of nitrogen excretion ($P<0.0001$). Serum pro-inflammatory (IL6 and IL8) and anti-inflammatory (IL10) cytokine levels were elevated in response to injury returning to normal levels 4–6 weeks post-injury. HMGB1 and mtDNA ($P<0.001$) in the serum of trauma patients were significantly elevated when compared to healthy controls.
These results provide evidence of the inflammatory and endocrine drivers of trauma-related sarcopenia and suggest that modifying the inflammatory and HPA axis response could reduce sarcopenia.

DOI: 10.1530/endoabs.34.S5.1

S5.2

Pituitary sequelae of traumatic brain injury

Chris Thompson
Beaumont, Dublin, Ireland.

Traumatic brain injury is well recognised to cause pituitary dysfunction. Although data suggests that this is uncommon after mild head injury (GCS 14–15), the consensus from a large number of papers, using different assays and methods of testing, is that 15–30% of long term survivors of TBI have pituitary dysfunction. This offers the option of medical therapy after TBI, to improve quality of life and, potentially, improve rehabilitation.
In the acute phase of TBI, it is clear that vascular insult to the pituitary can cause acute anterior hypopituitarism, in addition to hyponatreamia due to SIADH and diabetes insipidus. Recent evidence from our own group has shown that as many as 78% of patients will have inappropriately low plasma cortisol concentrations for a sick patient. Approximately 10% of post TBI 'SIADH' is also attributable to cortisol deficiency. Diabetes insipidus is a predictor of early mortality after TBI. Most acute hormonal changes improve spontaneously, but some persist to become chronic deficiencies. In addition, some patients with normal pituitary function can show deterioration to hypopituitarism over the 3–6 months after TBI. Changes are less dynamic thereafter, with most deficiencies permanent.
Chronic hypopituitarism varies from isolated pituitary hormone deficits to severe panhypopituitarism; most diabetes insipidus and nearly all SIADH recovers completely.

The challenge for screening is to identify the patients most likely to benefit from screening, given the logistics of patient numbers. Currently there is little consensus on the accuracy of predictors of long-term hypopituitarism, which can be utilised to form the basis of a functioning screening programme for post-TBI hypopituitarism.

DOI: 10.1530/endoabs.34.S5.2

S5.3

Thyroid hormone economy during critical illness

Anita Boelen
Academic Medical Center, Amsterdam, The Netherlands.

During illness changes in thyroid hormone metabolism occur, collectively known as the non-thyroidal illness syndrome (NTIS). NTIS is characterized by low serum thyroid hormone levels, while TSH and TRH expression do not increase, indicating a disturbance of the normal thyroid hormone feedback regulation.
Although the common view was that NTIS results in overall downregulation of metabolism in order to save energy, recent work has shown that genes involved in thyroid hormone (TH) metabolism show remarkably variable, illness-induced changes in key metabolic organs such as liver, muscle and adipose tissue, ranging from inhibition to activation. Organ- and timing-specific changes in the TH deiodinating enzymes (deiodinase types 1, 2, and 3) highlight deiodinases as proactive players in the response to illness. That illness-induced changes in peripheral organs appear to be very different during acute or chronic inflammation adds an additional level of complexity to the syndrome.
Recently, we showed that the macrophage is a novel and potentially important cell type involved in NTIS during acute inflammation. Disturbances in type 2 deiodinase, the T_3 activating enzyme affects phagocytosis and macrophage specific cytokine production. In addition, complex changes in thyroid hormone metabolism occur in muscle during critical illness that may be relevant for the pathogenesis of respiratory failure. Although acute NTIS may represent an adaptive response to support the immune response, NTIS may turn disadvantageous when critical illness enters a chronic phase necessitating prolonged mechanical ventilation, dialysis and inotropic support.
In sum, NTIS appears to be a timing-related and organ-specific response to illness, occurring independently from the decrease in serum thyroid hormone levels and potentially relevant for disease course.

DOI: 10.1530/endoabs.34.S5.3

Thyroid cell biology comes to your clinic (Supported by *Journal of Molecular Endocrinology*)

S6.1

Development of functional thyroid tissue from embryonic stem cells

Sabine Costagliola
IRIBHM Université Libre de Bruxelles, Bruxelles, Belgium.

Induced overexpression of defined transcription factors has been shown to have a directing effect on the differentiation of embryonic stem cells (ESCs) into specific cell types. Nevertheless, protocols promoting coordinated self-assembly of differentiated cells into distinct morphological units with functional properties reminiscent of organs and tissues *in vivo* are still very sparse. Our group recently reported efficient rescue of hypothyroidism in athyroid mice transplanted with functional thyroid follicles generated from mouse ESCs *in vitro*. In this work, we show that an overexpression of the transcription factors NKX2.1 and PAX8 is sufficient to direct ESCs differentiation into thyroid follicular cells (TFCs) and promotes *in vitro* self-assembly of TFC into three-dimensional follicular structures, when associated to a subsequent TSH treatment. Cells differentiated by this protocol show significant iodide organification activity, a hallmark of thyroid tissue function. Importantly, athyroid mice grafted with ESCs-derived thyroid follicles show normalization of plasma T_4 levels with concomitant decrease of plasma TSH. In addition, a full normalization of body temperature at 4 weeks after transplantation was observed. Together, these data clearly demonstrate that grafting of our ESCs-derived thyroid cells rescues the hypothyroid state and triggers symptomatic recovery along with the normalization of plasma hormone concentrations.
By using human pluripotent stem cells, our system would provide an unprecedented opportunity to improve our understanding of the molecular mechanisms underlying congenital hypothyroidism or to study papillary thyroid carcinoma risk allele under controlled *in vitro* conditions. Those thyroid diseases could be modelled, opening new therapeutic perspectives (Figure 1). One example could be the generation of functional thyroid tissue from induced pluripotent stem cells (iPSCs) derived from patients skin fibroblasts with the ultimate aim being the transplantation of iPSC-derived thyroid tissue to replace absent, ablated or damaged thyroid and restore an euthyroid state lifelong without a substitution therapy.

DOI: 10.1530/endoabs.34.S6.1

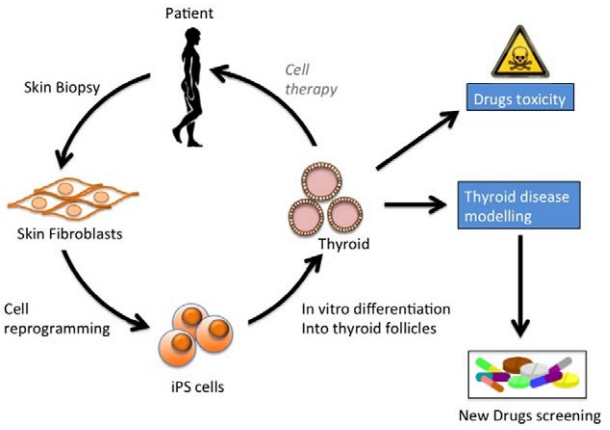


Figure 1

S6.2

Abstract unavailable.

DOI: 10.1530/endoabs.34.S6.2

S6.3

PBF signalling in thyroid tumourigenesis

Chris McCabe
University of Birmingham, Birmingham, UK.

Most of the oncogenic events in thyroid cancer are known, and the pathways which govern thyroid cell proliferation have been exquisitely mapped. However, existing knowledge does not fully explain the genetic complexity of thyroid cancer, or reveal whether tumours are likely to be benign, malignant, or resistant to treatment. Whilst mutations and chromosomal rearrangements are central to the aetiology of DTC, the altered expression of proto-oncogenes and tumour suppressor genes is likely to exert a critical effect on thyroid cell growth and behaviour. One proto-oncogene which appears to have a central role in thyroid cell physiology is the PTTG1-binding factor (PBF). Expression of PBF is elevated in DTC and correlates with early recurrence, whilst patients with high PBF expression show significantly reduced disease-specific survival. The peptide sequence of PBF shares no significant homology with other human proteins, but is highly conserved. A plethora of roles are emerging for PBF in thyroid cells, revealing that PBF binds to several proteins and regulates their subcellular distribution. Via this route, PBF represses the activity of the sodium iodide symporter NIS, which is essential to radioiodine treatment of thyroid cancer, and monocarboxylate transporter 8 (MCT8), which modulates thyroid hormone efflux from the thyroid. Our observations also point to critical roles in the regulation of p53 stability and cell invasion. Further, we have discerned that PBF is phosphorylated and glycosylated *in vitro* and *in vivo* in murine and human thyroid cells, implying post-translational modes of regulation. Although classically a proto-oncogene, 13 substitution-missense mutations of PBF have now been reported in tumour samples via the COSMIC database, suggesting PBF may in fact be capable of functioning as an oncogene. To this end, we and others are now beginning to define the roles of PBF in thyroid cell transformation, tumour progression and thyroid cancer treatment.

DOI: 10.1530/endoabs.34.S6.3

Natriuretic peptides: so much more than salt and water

S7.1

Guanylyl cyclases in cardiomyopathy

Michaela Kuhn
University of Wuerzburg, Wuerzburg, Germany.

Natriuretic peptides and their cGMP-synthesizing guanylyl cyclase receptors (GC-A, receptor for ANP and BNP; GC-B, for CNP) are critically involved in the regulation of arterial blood pressure (all NPs) and skeletal growth (only CNP). Both receptors are expressed in all cardiac cell types, although at different densities. GC-A is expressed at higher levels in endothelial and smooth muscle cells, whereas GC-B predominates in myocytes and fibroblasts. In heart failure (HF) patients, plasma levels of BNP and (less) ANP are elevated, correlating with the severity of the disease. However, despite these high NP levels, HF is in fact a state of combined deficiency of the active processed form of BNP and resistance to both NPs. Hence GC-A-mediated cGMP formation is markedly blunted, due to desensitization of the receptor. In contrast, GC-B activity is preserved. To study the specific cardiac impact of GC-A dysfunction, we generated various genetic mouse models with conditional, cell-restricted inactivation of this receptor. Our observations in these mouse models indicate that the NP/GC-A/cGMP system antagonizes the $Ca^{2+}{}_i$-dependent hypertrophic growth response of myocytes to angiotensin II but not to β-adrenergic stimulation. The selectivity of this interaction is determined by activation of cGMP-dependent protein kinase I and phosphorylation of specific proteins regulating or mediating Ang II/AT_1-signalling (RGS2, TRPC3/6 channels). In addition our studies show that BNP, produced by myocytes in response to pressure-load or hypoxia, promotes the regeneration of neighboring endothelia via GC-A. This paracrine communication might be critically involved in coordinating myocyte hypertrophy and angiogenesis. We conclude that GC-A desensitization, leading to an endocrine but also local, cardiac imbalance between NPs and Ang II/aldosterone can contribute to the progression from hypertrophy to heart failure.

Supported by the Bundesministerium für Bildung und Forschung (BMBF01 EO1004).

DOI: 10.1530/endoabs.34.S7.1

S7.2

Natriuretic peptides in pituitary development and function

Robert Fowkes[1], Samantha Mirczuk[1], Andrew Lessey[1], Victoria Lipscomb[1], Stijn Niessen[1], Craig McArdle[2] & Imelda McGonnell[1]
[1]Royal Veterinary College, University of London, London, UK; [2]LINE, University of Bristol, Bristol, UK.

The cardiovascular properties of the natriuretic peptide family of hormones are well-characterised, and provide valuable prognostic indicators in the treatment of heart failure in humans and companion animals. Of the three main mammalian natriuretic peptides, C-type natriuretic peptide (CNP) remains the most enigmatic, even though CNP represents the ancestral natriuretic peptide from which Atrial- (ANP) and B-type (BNP) natriuretic peptides evolved through gene duplication. We have focussed our studies on the role of CNP in the anterior pituitary gland and areas of the CNS, as these tissues represent major sites of expression of the peptide, and biological function, as established through pharmacological studies of the CNP-selective guanylyl cyclase B (GC-B) receptor. Using several biological models (humans, rodents, cell lines, Zebrafish and cats), we have started to establish the developmental profile of the CNP system in the pituitary, and have identified novel target genes downstream of CNP signalling. The genetically-tractable Zebrafish has proved an invaluable tool in the regard; we have cloned four separate CNP genes, each with subtly different spatiotemporal expression profiling, but all present within the CNS. We have developed multiplex RT-PCR assays to quantitate changes in these transcript levels, but also those of pituitary hormone markers, and found that exposure of developing Zebrafish larvae to exogenous CNP causes significant changes in pituitary hormone transcripts. Our *in vitro* studies, using gonadotroph-derived cell lines, suggest that components of the natriuretic peptide system are sensitive to pulsatile stimulation by gonadotrophin releasing hormone. Finally, our studies using astrocyte cell lines have revealed that CNP signalling is significantly inhibited by exposure to pathological concentrations of ammonia, mimicking conditions seen in patients with liver disease. These ongoing studies increase our understanding of the diverse role that CNP plays within neuroendocrine and CNS tissues.

DOI: 10.1530/endoabs.34.S7.2

S7.3

C-type natriuretic peptide and cardiovascular disease

Adrian Hobbs
Barts and The London SMD, QMUL, London, UK.

Natriuretic peptides are a family of vasoactive mediators that play fundamental roles in the regulation of blood volume, blood pressure and cardiac integrity. The physiological and pathological functions of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) in the context of cardiovascular homeostasis are well-characterised. There is much less clarity with respect to C-type natriuretic peptide (CNP) which, in contrast to its cardiac-derived siblings, is predominantly synthesised and released from vascular endothelial cells. Over the last 10 years, our work has focused on defining the key cardiovascular processes that are governed by endothelium-derived CNP in health and disease. Using both pharmacological and genetic interventions, a multi-faceted vasoprotective profile of CNP has been established, comprising regulation of vascular tone and local blood flow, maintenance of blood vessel morphology, the reactivity of leukocytes and platelets, and protection against ischaemia–reperfusion injury. In accord, loss of CNP bioactivity precipitates endothelial dysfunction, vasoconstriction and hyperreactivity of circulating cells. Thus, the paracrine effects of CNP, released by the vascular endothelium, appear to complement the endocrine activity of ANP and BNP to coordinate numerous aspects of cardiovascular physiology and pathology.

DOI: 10.1530/endoabs.34.S7.3

Chronic disease and growth

S8.1

Understanding GH action through the growth plate'

Colin Farquharson
University of Edinburgh, Edinburgh, UK.

The rate of bone growth is controlled by highly regulated activities of chondrocytes within the epiphyseal growth plate. Such activities allow rate of growth to function as a variable during postnatal development. Simplistically, growth rate is determined by the number of cells within the proliferative zone which is regulated by their rate of proliferation and also their rate of differentiation into the hypertrophic phenotype. In turn, a strong positive correlation exists between the final hypertrophic cell volume and the rate of growth. Interruption and/or deregulation of this highly ordered sequence of events results in impaired bone growth and short stature which, in a significant number of children, is not rectified by catch-up growth. Inhibitors of growth can act both exogenously (e.g. physical trauma and pharmacological agents) or endogenously (e.g. altered autocrine/paracrine and systemic control). Examples of the latter include alterations to the GH/IGF1 axis which is recognised to be a key pathway in the regulation of bone growth. Whilst the growth promoting role of GH on linear bone growth is well accepted the relative contributions to post-natal growth of the direct or indirect effects of GH remain unclear. The GH indirect effects are via endocrine and/or local (growth plate cartilage) IGF1 production. Various spontaneous mouse mutations in GH/IGF1 signalling have been informative. Also, the mouse genetic revolution with the creation of various transgenic and knock-out (inducible and tissue specific) strains of mice e.g. SOCS2 null mice, have helped us understand more fully the role of GH and IGF1 initiated pathways together with their negative feed-back loops and associated kinases and phosphatases in the linear bone growth process. Notwithstanding the direct effects of GH on growth plate chondrocytes it is likely that these two systems function in a highly coordinated manner to regulate growth plate function and linear bone growth. This presentation will summarise the current state of understanding and introduce emerging insights.

DOI: 10.1530/endoabs.34.S8.1

S8.2

Targeting SOCS proteins to combat inflammation

Stephen Yarwood
University of Glasgow, Glasgow, UK.

The SOCS3 gene encodes an E3 ubiquitin ligase component that targets inflammatory cytokine receptor components for proteolytic degradation. Our research in this area is aimed at determining the molecular control of SOCS3 gene induction by cyclic AMP in vascular endothelial cells (VECs) as a new route for anti-inflammatory drug action. We found initially that cyclic AMP activates the guanine nucleotide exchange factor, EPAC1, to mobilise C/EBP transcription factors to induce SOCS3 expression in VECs. Maximal SOCS3 induction in response to cyclic AMP also requires activation of JNK and ERK MAP kinase signalling pathways, which target AP-1, STAT and SP3 transcription factors on the minimal SOCS3 promoter. ERK and JNK activation by cyclic AMP occurs through novel protein kinase C-dependent mechanisms, independently of EPAC1 and protein kinase A. We have also found that some of these mechanisms can also be activated by bioactive small molecules, including citrus-derived flavanoids, which target activation of SP3 transcription factors. Our future work in this area will be aimed at further identifying small molecule regulators (e.g. PDE4 inhibitors) of these novel signalling mechanisms in VECs.

DOI: 10.1530/endoabs.34.S8.2

S8.3

Managing abnormalities of growth in chronic diseases

Dominique Simon
Hopital Robert Debré, Paris, France.

Growth retardation is often observed in patients suffering from chronic diseases. Factors involved in growth delay are multiple. They can be divided into two groups: those due to the disease itself and those due to treatments. Under nutrition is frequently reported in chronically ill children, due to an imbalance between decreased caloric intake and increased energy requirements. Malabsorption, dehydration, chronic anaemia, inflammation also impact growth, as well as steroid therapy. The most frequent abnormality in these patients is a decrease in IGF1 levels. Low IGF1 levels are associated with impaired GH secretion or with normal or high GH levels. Several mechanisms underlie the decrease in IGF1 concentration: a functional GH deficiency, a resistance to GH mediated and/or an increase in IGF1 clearance due to a raised proteolysis of IGF-BP3. Besides the systemic effects on GH–IGF1 axis, a direct effect of under nutrition, cytokines and steroid therapy on chondrogenesis has also been reported.

Assessment of growth and pubertal development should be performed by the endocrinologist. Biological tests can contribute to the understanding of hormonal abnormalities that might influence disturbances of growth and puberty. A tight collaboration between the endocrinologist and the clinician in charge of the disease is needed to evaluate the severity of the disease and the modalities of its treatment. The availability of more efficient treatment may allow for reducing the impact of the chronic disease on growth. However, GH therapy treatment and/or sex steroid should be considered as adjunctive therapies in case of severe growth failure and pubertal delay.

In conclusion, chronically-ill children are at high risk of developing growth failure. They should be followed by endocrinologists early in the course of their disease to detect growth disturbances. The later the patient will be seen, the less advantageous will be the long-term benefits of hormonal therapies.

DOI: 10.1530/endoabs.34.S8.3

MEN1 – from molecular pathology to therapies (Supported by *Endocrine-Related Cancer*)

S9.1

Current understanding and future perspectives in MEN1: the molecular pathology of the *MEN1* gene and menin

Sunita Agarwal
National Institutes of Health, Bethesda, Maryland, USA.

The *MEN1* gene is located on chromosome 11q13; it spans 9 kb, has ten exons, and encodes a 610 amino acid protein named menin (isoform-2, NCBI Reference Sequence: NP_570711.1). Germline heterozygous inactivating mutations in this gene are observed in 70% of patients with the multiple endocrine neoplasia type 1 (MEN1) syndrome who develop multiple tumours in various endocrine (parathyroid, pancreas, and pituitary) and non-endocrine tissues with loss of the remaining normal MEN1 allele. Also, a variety of MEN1-like non-familial sporadic tumours possess somatic biallelic inactivation/loss of MEN1. With more than 1000 *MEN1* mutations reported, significant hot spots and genotype–phenotype correlations are not obvious. Identification of this gene has facilitated genetic testing for germline *MEN1* mutations in at-risk individuals that allows early monitoring for tumours. Further investigations are required to elucidate the genetic causes of MEN1 in patients (30%) who lack an identifiable germline *MEN1* mutation. Menin is ubiquitously expressed and predominantly nuclear. Majority of the protein-truncating *MEN1* mutations disrupt the nuclear localization signals predicting functional consequences due to defective localization of menin. Thirty percent of *MEN1* mutations are missense. The recently deciphered crystal structure of menin can help to determine their consequence. Menin can undergo phosphorylation at Ser394, Thr397, Thr399, Ser487, Ser543, and Ser583. The importance of phosphorylated menin in normal and tumour cells should be further investigated. Mouse models, protein interaction studies, and gene expression analyses have been used to explore the critical biological function/s of menin. $Men1^{-/-}$ mice are embryonic lethal (E10.5–14.5), while the $Men1^{+/-}$ mice re-capitulate the human MEN1 syndrome. Menin is required in the MLL-complex for the epigenetic H3K4me3 chromatin modification. Anti-H3K4me3 ChIP-Seq in WT and $Men1^{-/-}$ mouse embryonic stem cells, and in *in vitro* differentiated pancreatic islet-like endocrine cells revealed menin-dependent expression of genes at the *Meg3* locus and at the four *Hox* loci. Interestingly, in MLL-fusion leukaemia menin-dependent expression of *Hox* genes is essential for the action of oncogenic MLL-fusions. Exploring this tumour-suppressing vs pro-oncogenic function of menin is of interest to identify druggable targets in MEN1-associated and similar sporadic tumours, and in MLL-fusion leukaemia. Thus, studies about the *MEN1* gene and menin can improve the management of MEN1-associated and sporadic tumours, and can help to develop new diagnostic and therapeutic options.

DOI: 10.1530/endoabs.34.S9.1

S9.2

Genetics, pathophysiology and translational models of MEN1

Rajesh Thakker
University of Oxford, Oxford, UK.

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterised by the occurrence of parathyroid, pancreatic islet, and anterior pituitary tumours. In addition, some patients may also develop adrenal cortical tumours, carcinoid, facial angiofibromas, collagenomas, and lipomatous tumours. The gene causing MEN1 is located on chromosome 11q13, and encodes a 610 amino-acid protein, menin, that represents a tumour suppressor, as its loss of expression is associated with the development of MEN1-associated tumours. *In vitro* studies have shown that menin has functions in cell division, genome stability and transcription regulation. In addition, menin acts as a scaffold protein and may increase or decrease gene expression by epigenetic regulation via histone methylation. *In vivo* mouse models for MEN1, established by using conventional and conditional knock-out methods, develop MEN1-associated tumours, and have been useful for translational studies. For example, one study has reported that MEN1-gene replacement therapy can reduce proliferation rates in anterior pituitary tumours. In conclusion, elucidation of the genetic defect causing MEN1 has helped to understand the pathophysiology of this disorder as well as establishing pre-clinical model for translational studies.

DOI: 10.1530/endoabs.34.S9.2

S9.3

Abstract unavailable.

DOI: 10.1530/endoabs.34.S9.3

Fat and bone – old neighbours, new relationships? (Supported by *Endocrine Connections*)

S10.1

Fat and its influence on bone

Jennifer Walsh
Academic Unit of Bone Metabolism, Sheffield, Sheffield, UK.

In general, higher body weight in adults is associated with higher bone mineral density and is protective against fracture, but may be associated with an increased risk of some fractures. Possible mechanisms for higher bone density include mechanostat response to increased loading and increased oestrogen production by adipocyte aromatase.

It has been increasingly recognised that bone interacts with other organs and tissues (such as fat, the gastrointestinal tract and the CNS), and also that fat is not just a passive energy reservoir but an endocrine organ with regulatory functions. Fat may have effects on bone through the actions of leptin in the CNS and directly on bone cells, and other adipokines such as adiponectin may also affect bone metabolism.

Subcutaneous and visceral fat may have differing effects on bone; visceral fat produces inflammatory cytokines which have pro-resorptive effects on bone, and higher visceral fat mass has been associated with increased bone turnover and lower bone density.

Intramuscular fat may also have effects on the skeleton, possibly through modulation of muscle loading.

There is generally an inverse relationship between bone density and the amount of bone marrow fat. It is not yet clear whether increased marrow fat contributes to the causation of osteoporosis, but models of osteoporosis with high marrow fat such as anorexia nervosa may offer insights into this relationship.

Understanding the inter-relationship of fat and bone can improve our understanding of the causes of osteoporosis and may offer new therapeutic targets.

DOI: 10.1530/endoabs.34.S10.1

S10.2

Obesity and fractures: what is the relationship?

Juliet Compston
University of Cambridge, Cambridge, UK.

Until recently obesity was believed to be protective against fractures as a result of higher bone mass and the cushioning effect of subcutaneous tissue during falls. However, recent studies indicate that fractures in obese postmenopausal women and older men make a substantial contribution to the overall fracture burden in this population. The effect of obesity on fracture risk is site-dependent, with some protection against hip and wrist fractures but increased risk of ankle and lower leg fractures. Risk factors for fractures in the obese are similar in many respects to those in the non-obese population, although increased risk of falling and reduced mobility are likely to play a prominent role.

The pathophysiology of bone fragility in obese individuals has not been clearly established. Visceral adipose tissue produces adipokines and cytokines, many of which have adverse effects on bone, and its presence is inversely related to bone mineral density. Increased intramuscular adipose tissue is likely to contribute to reduced muscle strength, reducing bone mass and increasing the risk of falls. Other potential pathogenetic factors include vitamin D insufficiency, secondary hyperparathyroidism and hypogonadism.

The evidence base for efficacy of anti-osteoporosis medication in the obese is weak, since pivotal clinical trials have included relatively few obese individuals. There is some evidence from *post hoc* subgroup analyses that anti-resorptive medications may not be effective in reducing non-vertebral fractures in obese postmenopausal women; however, whether this reflects inadequate dosing or other factors is unclear. Weight loss in association with exercise regimens may be beneficial. However, further studies are needed to enable the development of effective strategies to reduce the growing fracture burden in the obese population.

DOI: 10.1530/endoabs.34.S10.2

S10.3

Energy deficiency, amenorrhea and the skeleton: challenges and therapies

Mary Jane De Souza
Pennsylvania State University, University Park, Pennsylvania, USA.

The physiological adaptations associated with energy deficiency contribute to menstrual cycle disturbances. The downstream effects of both energy deficiency and hypoestrogenism synergistically impair bone health, leading to low bone mineral density, compromised bone structure and microarchitecture, and ultimately, a decrease in bone strength. Low bone mineral density is frequently observed among exercising women and anorexic women with functional hypothalamic amenorrhea (FHA) secondary to an energy deficiency. In these women with chronic energy deficiency, chronic hypoestrogenism also plays a significant role in the bone loss observed. Both amenorrheic athletes and anorexic women present with low BMD, decreased trabecular volumetric low bone mass and a deterioration of trabecular microarchitecture, indicating that the synergistic effects of an energy deficiency and estrogen deficiency impair bone quantity and quality, especially within trabecular regions. In the absences of a therapeutic intervention, 2–3% of bone loss per year is observed in these women. Therapeutic strategies to reverse bone loss in athletes and anorexic women with FHA and energy deficiency have included non-pharmacological approaches that target body weight restoration and resumption of menses. Several pharmacological strategies that include, combined oral contraceptives, transdermal estrogen, recombinant leptin and IGF1, and bisphosphonates have also been evaluated. The risks and benefits of non-pharmacological and pharmacological therapies to reverse bone loss in these energy deficient populations will be discussed. To date, the reversal of menses and restoration of body weight has resulted in the most promising improvements in bone health in both amenorrheic athletes and anorexic women.

DOI: 10.1530/endoabs.34.S10.3

Some like it hot – new insights into brown adipose tissue

S11.1

Molecular regulation of brown and beige fat cell fate

Patrick Seale, Wenshan Wang, Sona Rajakumari & Matthew Harms
University of Pennsylvania, Philadelphia, Pennsylvania, USA.

Brown adipocytes in dedicated depots of brown adipose tissue (BAT) arise from cellular precursors that also give rise to skeletal muscle cells. We found that early B cell factor-2 (*Ebf2*) expression in the mesoderm of mouse embryos marks brown fat precursors that are destined to activate Pparγ expression and differentiate into Ucp1+ brown adipocytes. Loss of Ebf2 in mice causes a very severe defect in BAT development and function. Ebf2 cooperates with Pparγ to activate expression of many brown fat-selective target genes, including *Prdm16*, a key transcriptional co-activator in brown adipocytes. Surprisingly, genetic loss of *Prdm16* specifically in the brown fat lineage does not disrupt embryonic BAT formation or the expression of brown fat-specific genes. Interestingly however, *Prdm16*-deficiency caused ectopic expression of white fat-selective genes and a severe aging-associated decline in the thermogenic characteristics of BAT. *Prdm16*-deficient BAT from older mice had reduced mitochondrial content and function. Moreover, animals lacking Prdm16 in BAT had a severely depressed capacity to increase their energy expenditure in response to β-adrenergic activators or cold. Importantly, BAT-selective deletion of *Prdm16* impaired BAT function but did not affect the thermogenic 'beige' adipocyte population in WAT; this serves as a model to distinguish the effects of brown and beige adipocytes in systemic metabolism.

DOI: 10.1530/endoabs.34.S11.1

S11.2

The metabolic capacities of brown adipose tissue

Barbara Cannon
Stockholm University, Stockholm, Sweden.

In rodents, brown adipose tissue is known to be a metabolically highly active organ. This concerns rates of blood flow, capacity for oxygen consumption and rates of uptake of lipid and carbohydrate substrates. The high rates of metabolism are dependent on stimulation of the tissue by norepinephrine, released from sympathetic neurons innervating the tissue. Following stimulation, thermogenesis occurs as a result of activation of the uncoupling protein UCP1 in the inner membrane of the numerous mitochondria. UCP1 is found only in brown adipocytes and in adipocytes with brown-like features, called brite (brown-like in white) or beige adipocytes, dispersed in several white adipose depots. Although the brite adipocytes appear attractive candidates to promote energy utilization and thus potentially weight loss, their natural abundance is markedly lower than that of classical brown adipocytes. The ability of brown adipose tissue to modulate body weight through UCP1-mediated thermogenesis, to dispose of whole body glucose and to clear triglycerides from the blood means that it has a capacity to improve many aspects of the metabolic syndrome. The confirmation of its activity in adult humans means that it is an interesting candidate for therapeutic development.

DOI: 10.1530/endoabs.34.S11.2

S11.3

How brown is human BAT?

Sven Enerback
University of Gothenburg, Gothenburg, Sweden.

Brown adipose tissue (BAT) was previously thought of as an animal-only tissue. Recently, this view was radically changed by several publications suggesting that active BAT might be part of normal human physiology. These results together with other papers on the same topic, make BAT-mediated dissipation of excess energy in humans a real possibility. Together, these advances are stimulating a radical reassessment of the role of brown adipose tissue in human pathophysiology. Furthermore, these new data have also opened up exciting new opportunities for the development of entirely new classes of therapeutics for metabolic diseases like obesity and type-2 diabetes. Any effective treatment for obesity must, over time, affect the total energy balance by either increasing expenditure or reducing intake. Complex hormonal, neuronal, genetic, and behavioral networks govern food intake and satiety. Research in these areas is focusing on new ways to combat obesity by modulating energy intake, a difficult approach necessitating long term neurological changes. The development of strategies to increase the amount and activity of human BAT provides an alternative and a conceptually attractive way to enhance energy expenditure, especially as this system has evolved with the sole purpose of safely dissipating large amounts of chemical energy.

DOI: 10.1530/endoabs.34.S11.3

Getting excited about sugar - the brain and blood glucose control (Supported by *Endocrine Connections*)

S12.1

Abstract unavailable.

DOI: 10.1530/endoabs.34.S12.1

S12.2

Vagal control of energy metabolism

Stefan Trapp
UCL, London, UK.

The activity of the vagus nerve is associated with a 'rest and digest' state, suggesting a central role in the regulation of energy homeostasis. The importance of the vagus nerve for cephalic responses to food intake, including the early release of insulin, is undisputed, and early experiments on rabbits by Claude Bernard almost 150 years ago have linked lesions in the dorsal vagal complex of the brainstem with diabetes mellitus. Similarly, many diabetic patients present with autonomic imbalance, and particularly a loss of vagal activity.

In our laboratory we focus on understanding this link between the vagal system and energy metabolism. We aim to ascertain the molecular mechanisms for assessing the metabolic state of the body at the level of the vagal complex**.** Using rodent *in vitro* brain slice preparations we investigate whether specific cell populations within the vagal complex are intrinsically sensitive to physiological changes in glucose concentration. We explore the molecular and electrical mechanisms responsible and the link which relates to their general metabolic sensitivity. We have successfully combined *in vitro* patch-clamp electrophysiology with single-cell RT-PCR and immunocytochemistry to demonstrate both metabolic and non-metabolic glucosensing pathways, as well as an element of circadian variation in glucose responses within the vagal complex. In parallel, we use optogenetic and pharmacogenetic approaches facilitated by viral gene delivery to manipulate vagal outflow specifically targeting the vagal complex *in vivo*. These experiments suggest that the level of vagal activity affects blood glucose regulation and also highlights a role for vagal activity in remote ischemic preconditioning of the heart.

In summary, we suggest that vagal output is important for metabolic regulation, and its loss may contribute causally to metabolic disease.

DOI: 10.1530/endoabs.34.S12.2

S12.3

Does glucagon need the brain for its effects on blood glucose?

Rory McCrimmon
University of Dundee, Dundee, UK.

The fundamental importance of insulin to glucose homeostasis is well recognized and most evident in type 1 diabetes where β-cell destruction and loss of endogenous insulin secretion lead to marked hyperglycaemia and ketosis. The pancreatic α-cell product, glucagon, has garnered less scientific attention. However, the demonstration that glucagon receptor null mice did not develop diabetes following near-complete chemical destruction of the pancreatic β-cell renewed interest in glucagon and its contribution to glucose homeostasis in health and disease. Therapies targeting glucagon secretion or action are currently undergoing clinical trials in both type-1 and -2 diabetes.

Glucagon stimulates hepatic glucose production through actions on gluconeogenesis and glycogenolysis. The liver is exquisitely sensitive to glucagon and hence any disruption glucagon secretion or actin has profound effects on glucose homeostasis. The regulation of glucagon secretion is complex and reflects the interplay between direct signalling molecules, intra- and extra-pancreatic signals and an extensive neural input. The relative importance of each of these modulatory signals is controversial. In this presentation, we will briefly discuss glucagon physiology before reviewing those intra-and extra-pancreatic systems that regulate glucagon secretion. We aim to address the question: 'Does glucagon need the brain for its effects on blood glucose?'

DOI: 10.1530/endoabs.34.S12.3

Applied Physiology Workshop (Supported by *Journal of Endocrinology*)

A guide to the analysis of energy metabolism

APW1.1

Assessing energy demand in living organisms: the influence of environmental temperature

Jan Nedergaard & Barbara Cannon
Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden.

The advent of mice as the most common animal used for metabolic studies was caused by the possibility to perform gene manipulations in this species (which first very recently has become possible in the previously most studied animal: the rat). Although the mouse in many ways would seem just to be a smaller version of the rat, the smaller size indirectly has added a confounding factor to interpretation of metabolic studies. The reason is that the environmental temperatures under which we study mice and rats are similar – but their thermoregulation is different. Temperatures around 20 °C are very cold for mice; they have to constantly increase their metabolism by some 50–75% in order to compensate for their heat loss (as compared to their metabolism at thermoneutrality, around 30 °C); for a rat, these temperatures are much closer to thermoneutrality. The extra heat production is generally caused by a recruitment and constant activation of brown adipose tissue. This constant extra heat production means that if any other thermogenic mechanism would be activated, the mouse would immediately diminish its brown-fat-derived heat so that there will be no metabolic effect of any alternative thermogenic mechanism; likely this phenomenon has led to several metabolic alterations being overlooked. Conversely, it would be realized that any change causing a decrease in e.g. skin or hair/fur appearance may appear as stimulating thermogenesis in a molecular way – whereas the increased heat production may just be 'physical', due to increased heat loss. Indeed, reports that activators of brown or brite/beige adipose tissue lead to increased metabolism and increased body temperature may be reporting the cause of brown/brite/beige adipose activation rather than the consequence. Due to the large effects on metabolism of this chronic cold exposure 'normal' mice endure, remarkable 'humanizations' of mouse metabolism may occur just by keeping mice at thermoneutrality.

DOI: 10.1530/endoabs.34.APW1.1

APW1.2

Lipid metabolism: from mice to men?

Samuel Virtue
University of Cambridge, Cambridge, UK.

The focus of this talk is on how disruptions in the ability of organisms to appropriately store and release lipid can impact on whole organism metabolic health. The talk focuses predominantly on murine phenotyping and discusses how observations from mice can be related to human data.

Both healthy mice and humans preferentially use carbohydrate in the fed state and switch to utilising a greater degree of lipid in fasted state. The ability to perform the switch between utilising carbohydrate and lipid can be defined as 'metabolic flexibility'. While metabolic flexibility has been extensively studied in humans, data from rodent models with alterations in metabolic flexibility are relatively rare.

The transcription factor peroxisome proliferator-activated receptor γ (PPARγ) is essential for adipogenesis. PPARγ has been implicated in the regulation of both carbohydrate and lipid metabolism and is therefore an excellent candidate for controlling metabolic flexibility. PPARγ2 is the adipose tissue-specific isoform of PPARγ and has greater transcriptional activity than PPARγ1. Despite the known role of PPARγ as a target for the thiazolidinedione class of anti-diabetic drugs, young mice (4 months of age) lacking PPARγ2 had surprisingly normal carbohydrate metabolism based on glucose and insulin tolerance tests.

In this talk, data from mice lacking PPARγ2 will be used to demonstrate how reductions in metabolic flexibility can be detected in mice using indirect calorimetry. The lower metabolic flexibility of the PPARγ2 KO mouse indicated that these animals may have impaired lipid handling, a phenotype which was subsequently confirmed; with PPARγ2 KO mice having reduced rates of lipolysis, and dramatically reduced capacity for whole-organism lipid clearance. Finally, the relationship between reduced adipose tissue capacity for both lipid uptake and release and human metabolic health will be discussed.

DOI: 10.1530/endoabs.34.APW1.2

APW1.3

Tracking lipid energy partitioning in human liver

Leanne Hodson
University of Oxford, Oxford, UK.

The liver is a relay station for fatty acid metabolism. Perturbations in hepatic fatty acid metabolism have the potential to impact widely on metabolic health. The accumulation or loss of liver fat (hepatocyte triacylglycerol (TG)) represents the balance between input pathways and removal pathways. In health these pathways must be closely balanced; imbalance will lead to changes in liver fat content. The concept of hepatic fatty acid partitioning is that fatty acids within the hepatocyte are broadly partitioned between two pathways: i) esterification to form mainly TG that will enter a hepatic TG pool and may be secreted as very low-density lipoprotein (VLDL)-TG, and ii) oxidation. In addition, the liver has the capacity for *de novo* lipogenesis (DNL).

Studying hepatic fatty acid partitioning in humans, *in vivo*, is challenging. Direct assessment of the liver can only be achieved by arterio-venous difference measurements, which is impractical in humans due to the inaccessibility of the portal vein. The use of stable isotope tracers and measurement of particles or molecules secreted by the liver such as VLDL-TG and 3-hydroxybutyrate (3-OHB) offers, at this point in time, the best insight into hepatic fatty acid metabolism in humans and have help advance our understanding.

Whether fatty acids are partitioned toward oxidation or esterification pathways appears to be dependent on a number of metabolic factors; not least ambient insulin concentrations. Insulin undoubtedly regulates the supply of fatty acids to the liver from adipose tissue, however, whether chronic hyperinsulinaemia has a direct intra-hepatic effect on hepatic fatty acid partitioning, in humans, remains unclear. Hepatic fatty acid partitioning in humans has been investigated in the postabsorptive and postprandial states. This talk will discuss how the synthesis and partitioning of fatty acids within the liver may alter with metabolic and nutritional state.

DOI: 10.1530/endoabs.34.APW1.3

Clinical Management Workshops

Workshop 1 (Supported by *Clinical Endocrinology*)
Management of complex obesity

CMW1.1

Have the new commissioning guidelines for bariatric surgery improved patient's pathways?

Julian Barth
Lees General Infirmary, Leeds, UK.

Bariatric surgery has become one of the fastest growing areas of medicine. However, the surgical procedure is only one step in the management of obesity. How should the clinical pathway for the obese individual be designed to ensure optimal results and provide the healthcare savings predicted by the health economic studies?
The Health and Social Care Act 2012 redesigned the NHS in England with local commissioning of clinical services and national commissioning for specialised services. The Specialised Commissioning Board has clinical reference groups including one for complex and severe obesity whose role is to design clinical service specifications.
The management of obesity, from prevention to bariatric surgery, is now structured in four tiers with bariatric surgery being in tier 4. However the four tiers are commissioned by different authorities. The obesity pathway, its' evolution and development will be discussed.

DOI: 10.1530/endoabs.34.CMW1.1

CMW1.2

Medical management of severe obesity

John Wilding
University of Liverpool, Liverpool, UK.

Obesity poses a major threat to health, increasing the risk of degenerative diseases and the burden of health costs. Those with severe and complex obesity (often with a BMI $> 40\ kg/m^2$) have the greatest burden of co-morbidity and reduced life expectancy are now 2% of the UK population. These patients comprise the majority of referrals to tier 3 obesity services in primary and secondary care. Bariatric surgery is recognised as an effective intervention for appropriate patients, but only a few thousand procedures were performed last year (public and private sector) for over a million severely obese in the UK, so the majority will require medical management, especially as this is also a prerequisite for surgery. Medical management requires assessment of causes and co-morbidities and offer of comprehensive support through a multidisciplinary team including doctors, dietitians, experts in physical activity, and psychological support. In some situations meal replacements or very low energy diets can be considered with appropriate monitoring and follow-up. At present pharmacotherapy is limited to the use of orlistat which can be of value to some; newer diabetes treatments such as GLP1 analogues and SGLT2 inhibitors may provide additional benefit to those with type 2 diabetes, but should not be used outside of this context.

DOI: 10.1530/endoabs.34.CMW1.2

CMW1.3

Endocrine and metabolic complications post-bariatric surgery

Nick Finer[1,2]
[1]University College Hospitals, London, London, UK; [2]National Centre for Cardiovascular Prevention and Outcomes, UCL Institute of Cardiovascular Science, London, UK.

The growing adoption of bariatric surgery (BS) as treatment for severe and complex obesity, is resulting in the emergence of both iatrogenic and nosohypokatastatic (disease substitution) diseases. Malabsorption (an integral part of gastric bypass surgery) may compound mineral, vitamin and trace element deficiencies that also result from poor nutrition that can be worsened by the restricted intake imposed by BS. Thiamine deficiency (especially in those who have frequent vomiting) can produce Wernicke encephalopathy. Vitamin D deficiency affects most patients with morbid obesity before BS. Bone loss is likely to occur after BS and fracture risk is increased, although possibly not related to the surgery *per se*. There is particular concern of the impact of BS in women of childbearing age who subsequently become pregnant. While BS is it is protective against infant macrosomia in obese mothers, it has been associated with multiple negative maternal and fetal outcomes. While one of the major benefits of bariatric surgery is to improve glycaemic control in patients with diabetes, with marked reductions in A1c even after withdrawal of hypoglycaemic therapy, it does not normalise blood glucose profiles. The significance of the (inevitable) excessive rises in post-prandial glucose levels to long-term micro- and macrovascular disease is unclear. However, an increasingly recognised metabolic consequence is 'reactive' hypoglycaemia that appears to result from the hyper-responsiveness of incretin response to meals. There continues to be debate as to whether nesidioblastosis or insulinoma can result from the continued exposure of the β-cell to incretins, but if this does occur it is rare. Some evidence points to an acquired hypothalamic unresponsiveness to hypoglycaemia as a contributory factor. Increased absorption of calcium oxalate can lead to deposition in the renal parenchyma, resulting in oxalate nephropathy and renal failure as well as an increased incidence of ureteric renal stones.

DOI: 10.1530/endoabs.34.CMW1.3

Workshop 2 (Supported by *Endocrinology, Diabetes & Metabolism Case Reports*)
How Do I Do It?

CMW2.1

Abstract unavailable.

DOI: 10.1530/endoabs.34.CMW2.1

CMW2.2

How and when do I initiate treatment for central hypothyroidism?

Luca Persani[1,2]
[1]University of Milan, Milan, Italy; [2]IRCCS Istituto Auxologico Italiano, Milan, Italy.

The central hypothyroidism (CeH) is a hypothyroid condition due to an insufficient stimulation by TSH of an otherwise normal thyroid gland. CeH is about 1000-fold rarer than primary hypothyroidism (PH). Differently to PH, the CeH is most frequently characterized by low/normal TSH levels and thyroid hormone replacement is associated with the suppression of residual TSH secretion even during low thyroxine regimen.
Thus, CH management often represents a clinical challenge because physicians cannot rely on the systematic use of the reflex TSH determination which complicates the diagnosis of milder or subclinical CeH forms and the fine tuning of replacement therapy to the individual needs. The clinical challenge of CH is further amplified by the frequent combination with other pituitary deficiencies that may mask the CeH manifestations. In the presence of hypothalamic/pituitary lesions, the replacement of gonadotrope or somatotrope functions can in turn uncover a subclinical or partial thyrotrope defect.

DOI: 10.1530/endoabs.34.CMW2.2

CMW2.3

Abstract unavailable.

DOI: 10.1530/endoabs.34.CMW2.3

CMW2.4

How do I investigate and manage sweating

F M Swords[1,2]
[1]Norfolk and Norwich University Hospital, Norwich, UK; [2]Norwich Medical School, University of East Anglia, Norwich, UK.

This practical and interactive session will explore the difficult clinical presentation of sweating.

This problem is very commonly encountered in primary care, and can present a major diagnostic and management challenge. In practise, although there are multiple endocrine causes for excessive sweating, in the majority of cases presenting with this symptom alone, no endocrine cause is found.
An overview of the most important aspects of history taking and examination will be given, to help identify patients in whom organic endocrine pathology is most likely.
An algorithm will also be presented to ensure that investigation is focussed and pragmatic. Multiple clinical case examples will then be presented to demonstrate the most common endocrine and non endocrine causes of sweating.
Finally, the session will summarise current best available management for patients with problematic hyperhydrosis, where endocrine pathology has been excluded.

DOI: 10.1530/endoabs.34.CMW2.4

CMW2.5

Abstract unavailable.

DOI: 10.1530/endoabs.34.CMW2.5

CMW2.6

How I do: hyponatraemia

Stephen Ball
Newcastle University, Newcastle, UK.

Hyponatraemia (serum $Na+ < 135$ mmol/l) is common. It is associated with increased mortality and morbidity across a range of clinical contexts. Despite this, it remains an area in which there is diverse practice. There are centre and speciality-specific approaches to diagnosis and management that reflect both the apparent absence of a clear evidence-base and differences in perceived clinical priorities.
This presentation will focus on a number of key themes.

An evidence-based approach to defining aetiology of the hyponatraemia.
Finding the balance in treating the patient rather than simply treating the serum Na+ concentration.
When (and when not) to use hypertonic fluid.
Approaches to the management of over-correction.

DOI: 10.1530/endoabs.34.CMW2.6

CMW2.7

Abstract unavailable.

DOI: 10.1530/endoabs.34.CMW2.7

Workshop 3 (Supported by *Clinical Endocrinology*) Improving the care of young people in endocrinology

CMW3.1

Adolescent health care in the UK in 2014

Janet McDonagh
University of Birmingham, Birmingham, UK.

In spite of the first adolescent health clinic being established in Stanford in 1918, it is only in the last decade that specific national standards, professional societies and training in adolescent health have been developed and established in the UK. Over this time, the World Health Organization (WHO) has declared the health of young people a global health priority, concerned both by the growing burden of adolescent morbidity and mortality and the importance of adolescence for lifetime health. Adolescents are a group for whom improvements in outcomes have not matched those seen among other age groups, even in high income countries. Whereas the positive benefits of investment in the early years are no longer in doubt, investment in young people's health is called for in order to consolidate such success. This is particularly pertinent in view of the increasing awareness of the adolescent predictors of adult health. Young person friendly health service provision in the UK is not yet universal. Current evidence provides a useful framework for the development of such services irrespective of setting. Important components of this framework include developmentally appropriate environments, health outcomes as well as such aspects as staff attitude, communication, medical competency, accessibility, guideline driven care and youth involvement. With the knowledge that brain development extends into the third decade, there is also growing interest in the potential benefits of adolescent and young adult (AYA) health care, as already modelled by disciplines such as oncology. This is a key area for effective advocacy from both paediatric and adult health care professionals. This presentation will aim to present the current state of play of adolescent health care provision in the UK in 2014 and the importance of taking a life course approach in future research and service development.

DOI: 10.1530/endoabs.34.CMW3.1

CMW3.2

Adrenal replacement in adolescents and young adults

Peter Hindmarsh
University College London, London, UK.

Classical congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency leads to glucocorticoid and mineralocorticoid deficiency. Management should be viewed as a process of care which requires input from an Inter-disciplinary Team. Glucocorticoid therapy should take the form of hydrocortisone in a starting dose of 10–12 mg/m^2 per day (divided into three or four doses) and the dose should be titrated to blood or urine profiles of cortisol and 17 hydroxyprogesterone. Mineralocorticoid replacement (9α fludrocortisone) requires higher doses in infancy and childhood compared to adolescence. Starting dose should be 150 μg/m^2 per day and thereafter titrated to plasma renin activity and blood pressure. Despite adequate glucocorticoid substitution and concordance with medical therapy, control can be difficult during puberty due to alterations in the clearance of hydrocortisone and dosing schedules may need to be adjusted to account for this. GH and IGF1 along with changes in sex steroid concentration influence the conversion of cortisol to cortisone through 11β hydroxysteroid dehydrogenase type 2. Twenty-four hours plasma cortisol and 17 hydroxyprogesterone profiling is a useful method for determining dose and frequency of hydrocortisone administration to avoid over and under treatment. Follow-up should address the many facets of CAH which should be assessed at an annual review and a suggested protocol is presented. A case for an extension of adolescent follow-up to cover the age range 19–25 years will be made.

DOI: 10.1530/endoabs.34.CMW3.2

CMW3.3

Optimising bone health in adolescents and young adults

Nick Shaw
Birmingham Children's Hospital, Birmingham, UK.

Childhood and adolescence represents an important period for accrual of bone mass and therefore changes during this time have a potential influence on adult bone mass and risk of osteoporosis. It is known that between 33 and 46% of adult bone mineral content is accrued over a 4-year period of adolescent growth surrounding peak height velocity. Although genetic factors are the most significant influence, environmental factors such as nutrition, exercise including mechanical loading, and puberty are critical influences with disturbances in these compromising bone density and strength leading to a risk of osteoporotic fractures. The assessment of bone density in children and adolescents is primarily undertaken with the use of dual energy X-ray absorptiometry (DXA) for which there is the availability of normative reference data for UK children. However it is recognised that factors in addition to bone density such as the material property of the bone and bone geometry are important determinants of bone strength which are not captured by DXA. Other imaging modalities are therefore being explored which provide this information. Chronic diseases and/or their treatment occurring

during childhood and adolescence can compromise bone strength leading to osteoporotic fractures in either the axial or appendicular skeleton. Inflammatory conditions such as juvenile idiopathic arthritis and crohn's disease either alone or in conjunction with corticosteroids often compromise trabecular bone in the spine with the potential development of vertebral fractures. Boys with Duchenne muscular dystrophy are at risk of vertebral and long bone fractures due to a combination of corticosteroid treatment and the progressive loss of mobility. The development of hypogonadism in conditions such as thalassaemia major can compromise bone strength if appropriate and timely hormone replacement does not occur. Thus the management of many of these conditions often requires an assessment of bone health to optimise skeletal development during childhood growth.

DOI: 10.1530/endoabs.34.CMW3.3

Workshop 4 (Supported by *Endocrinology, Diabetes & Metabolism Case Reports*)
Cardiovascular risk in endocrine disease

CMW4.1

Cardiovascular risk in Turner's syndrome

Malcolm Donaldson
School of Medicine, Glasgow University, Glasgow, UK.

The cardiac problems encountered in girls and women with Turner's syndrome include: congenital anomalies such as biscuspid aortic valve (30%) which is a risk factor for aortic stenosis and for future aortic rupture, coarctation of the aorta (12%), persistent left superior vena cava (13%) and partial anomalous pulmonary venous return (13%); a tendency towards hypertension (seen in 25% of adolescents) which appears essential in nature and which is not correlated with alterations in renal morphology (such as duplex and horsheshoe kidneys); aortic root dilation which may proceed to aortic rupture or dissection; and an increased incidence of atherosclerosis.

A thorough clinical examination of the cardiovascular systems at the time of diagnosis is essential, supplemented by an echocardiogram. Since preschool girls may be fearful, and hence difficult to assess, echocardiography should be repeated at the time of school entry.

Cardiovascular risk should be reduced by optimising blood pressure (BP) and preventing obesity during the childhood and adolescent years. BP should be measured with Dinamap annually up to school entry and then at each clinic visit. If systolic and/or diastolic blood pressure is $>$98th centile on more than one occasion then 24-h ambulatory blood pressure monitoring is indicated, to distinguish between labile hypertension related to anxiety; and sustained hypertension with failure of nocturnal dipping, which may need antihypertensive treatment (e.g. with Ca^{++} channel or β blockers). BP status may also be improved by using physiological induction and maintenance of puberty with transdermal rather than oral oestrogen.

At time of transfer to an adult endocrine or gynaecology clinic the heart and great vessels should be reassessed using MRI, which is superior to ultrasound in the detection of cardiac abnormality, including biscupid aortic valve. Aortic root dimensions are best expressed as aortic size index (ASI), to allow for the short stature. Adult management includes attention to obesity, hyperlipidaemia and BP, with regular cardiology follow-up[1]. Particularly careful surveillance is needed in order to pre-empt aortic dissection in pregnancy, and in women with ASI >2 cm/m^2.

Reference

1. Turtle *et al.* Cardiovascular risk in adults with TS. *Clin Endocrinol* 2013 **78** 639–645.

DOI: 10.1530/endoabs.34.CMW4.1

CMW4.2

Valvular disease in prolactinomas: weighing up the evidence

Will Drake
QMUL, London, UK.

Concern exists in the literature that the long-term use of ergot-derived dopamine agonists (DAs) may be associated with the development of clinically-significant cardiac valvulopathy. This has arisen largely by extrapolation from experience with high doses of these drugs given to patients with degenerative neurological conditions and it has led to regulatory authority recommendations to arrange regular screening echocardiograms for patients receiving much lower doses of these drugs for hyperprolactinaemic states. Many physicians experienced in the management of pituitary disease were surprised at those recommendations and adherence to the suggested protocols has been heterogeneous across the UK.

Addressing this issue by means of clinical research studies performed within endocrine clinics is not straightforward for a number of reasons. First, population control data on the incidence of cardiac valvulopathy in healthy individuals are surprisingly hard to find. Second, many of the single centre reports of screening echocardiograms performed in patients with lactotroph pituitary tumours treated with DA have involved sample sizes that make meaningful statistical analysis difficult. Third, echocardiography is a technique that relies on subjective as well as objective assessment and it is well-established in the field that knowledge of the clinical background and medication history can influence the findings of even experienced cardiac technicians.

This talk will summarise the published evidence on this important clinical practice point and attempt to draw useful, pragmatic conclusions for physicians practising in the field.

DOI: 10.1530/endoabs.34.CMW4.2

CMW4.3

Cardiovascular risk in childhood cancer survivors

Elizabeth Crowne
University Hospitals Bristol Foundation Trust, Bristol, UK.

Advances in childhood cancer treatment have led to an overall 5 year survival rate of almost 80%. As a result 1 in 900 adults are now childhood cancer survivors but have increased risk of long-term health consequences. Data from the USA, UK, and Europe have demonstrated a standardised mortality rate of 8.4–10.8. Cardiovascular morbidity (congestive heart failure, myocardial infarction, and strokes) have been reported as the commonest non-neoplastic causes of mortality, with tenfold higher risk than in siblings. Features of the metabolic syndrome are common among childhood cancer survivors even before adulthood. The length of time post oncology treatment, independent of the patient's chronological age, demographic background and BMI, is related to increased risk of metabolic abnormalities. Particular high cardiovascular risk is reported in childhood cancer survivors of brain tumours and leukaemia treated with cranial irradiation and/or bone marrow transplantation and total body irradiation (BMT/TBI). Possible contributing factors include direct chemotherapy and radiotherapy toxicity; endocrinopathies such as GH deficiency and gonadal failure; adipose tissue dysfunction and physical inactivity. Associations between metabolic abnormalities and abnormal body composition have been described. BMT/TBI is particularly associated with increased cardiovascular risk with abnormalities in body composition (increased visceral and intramuscular fat, reduced subcutaneous fat and lean mass), dyslipidaemia and increased diabetes risk. In two recent studies, we have demonstrated reduced β-cell reserve in addition to increased insulin resistance contributing to abnormal glucose tolerance, but improved fitness and insulin resistance after an exercise intervention in survivors of BMT/TBI. Current data in survivors of childhood cancer would indicate that screening and treatment of modifiable risk factors should be addressed at a much earlier age than currently recommended for the general population. Further studies into the prevention and management of cardiovascular risk in young adult survivors of childhood cancer are required.

DOI: 10.1530/endoabs.34.CMW4.3

Meet the Expert Sessions

MTE1

Abstract unavailable

DOI: 10.1530/endoabs.34.MTE1

MTE2

Vitamin D replacement: how much, how often and for whom?

Peter Selby
University of Manchester, Manchester, UK.

The high prevalence of vitamin D deficiency has increasingly become recognised as a problem for patients living in temperate regions. There has been considerable controversy regarding the optimal level of vitamin D nutrition and the means by which vitamin D should be replaced. The National Osteoporosis Society with the support of several other national groups including the Society for Endocrinology prepared guidelines on the diagnosis and management of vitamin D deficiency which were published in 2013. They are available for download at http://www.nos.org.uk/document.doc?id=1352.

The guidelines recommend that vitamin D status be assessed by measurement of serum 25 hydroxyvitamin D (25OHD) and that the following thresholds be applied:

- 25OHD <30 nmol/l=deficiency
- 30 < 25OHD <50 nmol/l=insufficiency, may be inadequate
- 50 nmol/l <25OHD=sufficient for almost all people

It is recommended that vitamin D status should be checked in the following groups of patients:

Patients with diseases that may be improved by vitamin D treatment.

Patients with musculoskeletal symptoms that could be attributed to vitamin D deficiency.

In asymptomatic individuals at high risk of vitamin D deficiency the current guidance from the Department of Health is commended but routine vitamin D screening in asymptomatic healthy individuals is not recommended.

The clinical consequences of these recommendations together with their practical application in the management of patients in clinic will be illustrated together with appropriate strategies for vitamin D replacement.

DOI: 10.1530/endoabs.34.MTE2

MTE3

Richard Quinton[1,2]
[1]Institute for Genetic Medicine, Newcastle University, Newcastle-Upon-Tyne, UK; [2]Endocrine Unit, Newcastle-Upon-Tyne Hospitals NHS Foundation Trust, Newcastle-Upon-Tyne, UK.

Klinefelter syndrome (KS) is defined by at least one extra X chromosome in the male karyotope. The phenotype is highly-variable, but a degree of progressive testicular dysfunction is universal. 47XXY is the most common sex chromosome aneuploidy in humans and, together with much rarer forms (e.g. 48XXY and 48XYY) occurs in 0.1–0.2% live male births. However the prevalence of diagnosed KS among adult males in the UK and elsewhere is much lower, indicating only around 25% of cases being identified. This isn't just a matter of poor diagnostics and awareness, but reflects the paucity of clinical signs and symptoms among the 'silent majority' of KS men.

Patient management falls into five broad categories, comprising i) post-diagnostic psychological support/counselling, ii) fertility options, iii) androgen replacement (ART), both in respect of maintenance therapy and optimal timing for its initiation, iv) gynaecomastia-surgery and v) long-term management of other disease risks, principally obesity/T2DM, but also including cancer (testes, breast, NHL, possibly lung), aortic valve and thromboembolic disease. These issues may overlap and their sequence will depend upon patient characteristics, stage of life and social considerations.

Contrary to the belief systems of many, ART is neither a common cause for 'excursions' in mental health status, nor a 'cure all'. Nevertheless, it is incumbent upon Endocrinologists to manage it efficiently and well, with maintenance of physiological haematocrit and bone density being key outcome measures. Depending on Iocal Andrologist practice, it might be best to defer ART microTESE is being actively planned.

In the UK, axis to psychological support requires close liaison with primary care, but it is important to remember the common themes expressed by patients and those close to them, namely: social isolation, lack of self-worth, and depression (including all five phases of the grief response, but perhaps with particular issues of anger-management).

DOI: 10.1530/endoabs.34.MTE3

MTE4

Abstract unavailable

DOI: 10.1530/endoabs.34.MTE4

MTE5

Abstract unavailable

DOI: 10.1530/endoabs.34.MTE5

MTE6

Abstract unavailable

DOI: 10.1530/endoabs.34.MTE6

MTE7

Who should the endocrinologist ask the ophthalmologist to review?

Daniel Ezra
Moorfields Eye Hospital, London, UK.

Thyroid eye disease (TED), or Graves' orbitopathy, is a common manifestation of autoimmune thyroid disease, affecting up to 30% of patients. Prompt identification of the development of TED is often difficult as the underlying inflammation of the orbital tissues and can lead to a wide range of symptoms and signs.

These include extraocular muscle fibrosis leading to diplopia, orbital congestion leading to optic nerve compression and lid retraction leading to corneal exposure. In addition, orbital fat hypertrophy and hyaluronic acid deposition can lead to periocular puffiness and significant disfigurement. Increased orbital pressure can also lead to glaucoma and chronic ocular surface irritation can be the underlying cause of periocular dystonias.

This wide variety of clinical phenotypes along with the inadequate correlation between current clinical scoring systems and disease activity can often make TED difficult to identify in the early stages. This can lead to delays in referral with significant patient morbidity.

In addition, the recent expansion of multicenter clinical trials programmes across the UK require patient with active TED for recruitment.

This lecture will focus on the different clinical presentations of TED to ensure that referral can be made in appropriate time. An up-to-date overview of the research landscape for TED trials in the UK will also be discussed.

DOI: 10.1530/endoabs.34.MTE7

MTE8

Abstract unavailable

DOI: 10.1530/endoabs.34.MTE8

MTE9

Novel techniques to assess angiogenesis

S Germain
Collège de France, Paris, France.

Promotion of angiogenesis, the formation of new blood vessel from preexisting blood vessels, is the focus of intense research and therapeutic efforts. In contrast, inhibition of endothelial cell growth and survival is a strategy to target many proliferative diseases. Various studies have shown that angiogenic growth factors are produced and secreted by normal endocrine cells and are increased in pathological states, including inflammation and hyperplasia. Importantly, angiogenesis plays a key role in the development of neoplastic disease which affects a number of endocrine glands.

In this paper, I will present the methods to study angiogenesis *in vitro* and *in vivo*. I will include the results from current studies on the role of angiogenesis factors that ultimately regulate the mechanical properties of the vascular milieu, matricellular protein expression and function and the availability of angiogenesis-regulating growth factors such as Vascular Endothelial Growth Factor (VEGF). In addition, I will discuss current and novel targets for inhibiting angiogenesis which could be exploited to develop new and improved therapeutic approaches for the treatment of endocrine neoplastic disease.

DOI: 10.1530/endoabs.34.MTE9

Education Workshops

Teaching and learning in Endocrinology

EW1.1

Endocrinologists as teachers: integrating teaching and practice in the real world

Karim Meeran
Imperial College, London, UK.

Endocrinologists are ideal teachers of physiology and biochemistry, major aspects of the underpinning of the science behind clinical practice. There are many methods that are successful in imparting this core knowledge to our medical students. Patients with endocrine disease also display symptoms and signs that that give clues to diagnoses, and the subject is ideal to serve as a broad general medical education.

Evidence that teachers are effective comes from exam performance, although student feedback is becoming increasingly important to the good teacher. Boehler *et al*. have demonstrated that feedback does not correlate with knowledge. Students were randomised to receive either constructive feedback on their performance, or just compliments, on their ability to tie surgical knots. Unsurprisingly, the group that were given constructive feedback improved their ability to tie knots significantly (performance score improved from 15.87 to 21.98 on knot tying) compared to the group who got only compliments (whose performance score went from 15.39 to 17.00). However when the students were asked to give feedback on their experience (as they are in the NSS), the group who were given praise (and hence did not really learn) were significantly more satisfied with their teaching (score 6.00) than those who had improved through feedback (satisfaction score 5.00); $P = 0.005$.

Student satisfaction is not an accurate measure of the quality of feedback. It appears that satisfaction ratings respond to praise more than feedback, while learning is more a function of feedback.

Impact factors and the Research Assessment exercise mitigate against good educational research, which is published in journals of low impact. The result of this is that universities tend to give educational research a low priority. Publishing ones educational research in specialist endocrine or other journals is one way of integrating teaching, clinical practice and research.

DOI: 10.1530/endoabs.34.EW1.1

EW1.2

Using technology to enhance your teaching in Endocrinology

Stephen Ball
Newcastle University, Newcastle, UK.

The process of learning is an order-dependent process. It can be broken down into functional components: accessing information; assimilating that information as knowledge within a theoretical framework; and then being able to apply that knowledge in an appropriate context. There is nothing new in this. Indeed, the process of learning is part of our very nature. Generations of doctors have learned and taught; learned and taught some more; and learned and taught some more after that.

So what's new? In 2014 and beyond, information and web-based technologies have ensured that the learner is no longer dependent on the teacher to facilitate access to information. Instead, we have a new set of problems. These include quality assuring the information available to those wishing to learn while, in parallel, developing their critical appraisal skills so they can (in time) become independent. Moreover, this has to be achieved in a time and resource-limited working-training environment, while balancing other commitments. Don't worry. Technologies are available to help.

This presentation will focus on the how best to harness local, national and international resources to support learning in Endocrinology, highlighting: social media; post-peer review repositories; and evolving e-learning platforms. Keep calm… and switch on.

DOI: 10.1530/endoabs.34.EW1.2

EW1.3

Assessment in Medicine and Endocrinology

Jane Dacre
UCL, London, UK.

Medical Education as an area of scholarship has been gathering a head of steam over the past 10 years. Academic colleagues have been increasingly interested in developing the evidence base with which to support innovations in medical education.

One of the areas which lends itself well to research is examinations and assessments of students and trainees.

This presentation will provide some background theory about current best practise in assessments, and will provide some of the research evidence that underpins these theories.

In medicine and endocrinology, assessments begin in the undergraduate years, and develop through medical school finals.

The UK training programme for physicians and endocrinologists is based on a mixture of formative and summative assessments. These will be outlined, with descriptions, and also data from the undergraduate course at UCL, and the MRCP (UK) examination.

The presentation will cover the latest issues which are influencing the way we assess our students and trainee doctors.

DOI: 10.1530/endoabs.34.EW1.3

EW1.4

Interprofessional learning through and for multidisciplinary working

Della Freeth
Queen Mary University of London, London, UK.

Modern healthcare requires effective multidisciplinary working: it is fundamental to modern professionalism, helps to ensure patient safety, supports efficient use of resources, and underpins patient safety. Through the mediating effect of improving the quality of working lives, it may also reduce burnout and staff turnover. So how do practitioners learn to collaborate effectively and to challenge poor collaboration? I will examine the evidence base for interprofessional collaboration (IPC) and interprofessional education (IPE) and, mainly drawing upon examples from my own IPC/IPE research programme, I will present insights into the nature of interprofessional learning through and for multidisciplinary working. Many examples will focus learning through simulated professional practice with the objective of enhancing patient safety. I define simulation broadly to encompass a spectrum from very simple role play activities or rehearsal of basic psychomotor tasks, right through to high fidelity multidisciplinary simulations in real or simulated clinical environments. I will introduce the educational concept of disjuncture and argue that debriefing is as important as the simulation itself (in fact, possibly more important).

DOI: 10.1530/endoabs.34.EW1.4

Practical publishing advice

EW2.1

Abstract unavailable.

DOI: 10.1530/endoabs.34.EW2.1

EW2.2

Abstract unavailable.

DOI: 10.1530/endoabs.34.EW2.2

EW2.3

Abstract unavailable.

DOI: 10.1530/endoabs.34.EW2.3

EW2.4

Abstract unavailable.

DOI: 10.1530/endoabs.34.EW2.4

EW2.5

Responding to reviewer comments

Stephen Ball
Newcastle University, Newcastle, UK.

Preparing and submitting a paper is a lot of work: designing the study; collecting the data; analysing the results; writing the manuscript. Then, depending on your supervisor or co-workers, writing the manuscript again. The last thing anyone wants is rejection. Even when accompanied by positive comments and useful steers on how best to improve things, it can feel as though the peer review process is not fair. Put simply, the world does not understand. While this may be true, it is often a distortion.
Ultimately, there is mutuality in the relationship between author and Journal editor. Managing the relationship is key. Authors want their quality work to be published while editors want to publish quality work. Most colleagues involved in peer review are reasonable, even though this may not appear to be the case in the heat of your initial reaction to their judgements.
This talk will focus on how to respond to the peer review process: giving examples of how to approach problems without escalating conflict; and how to engage (constructively) with apparent criticism. There are some basic rules that are good to know.

DOI: 10.1530/endoabs.34.EW2.5

EW2.6

The art of scientific writing

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

Good writing and communication skills are essential in many areas of science and medicine. Good scientific writing can help you with recording your observations, structuring your thinking and organising and explaining your work. Most scientists live in a 'publish or perish' environment, but few describe themselves as brilliant (or enthusiastic) writers. In this short talk within a larger session on writing and publishing, we will think about why good and clear writing is important to readers and to journal editors and we will consider how to write in a way that is appropriate, concise, engaging and easy to follow.

DOI: 10.1530/endoabs.34.EW2.6

EW2.7

Abstract unavailable.

DOI: 10.1530/endoabs.34.EW2.7

Nurse Session

Cushing's Syndrome

N1.1

Investigating and diagnosing Cushing's syndrome: not as straightforward as it seems?

Karim Meeran
Imperial College, London, UK.

Cushing's syndrome is rare at about 20 per million of population each year. Patients with Cushing's syndrome complain of increased weight, high blood pressure and diabetes. Obesity not caused by Cushing's is becoming very much more common, and obesity itself can also cause high blood pressure and diabetes. Distinguishing common simple obesity from rare Cushing's disease can be difficult. Patients with simple obesity are now able to surf the internet, and find forums about obesity and Cushing's syndrome. Some patients with Cushing's syndrome correctly put the nightmare of their story onto such discussion forums. The diagnosis may not have been considered for some time. They complain that no one thought of the diagnosis, and are annoyed with doctors, nurses and any other medical staff that they feel should have picked it up.

Patients with simple obesity who do not actually have Cushing's, but are desperate to lose weight might believe they actually have Cushing's, especially if they also have hypertension and diabetes. Large numbers of such people ask for the tests for Cushing's disease, and some might be convinced that their Cushing's has been missed, when in fact they don't have it at all, but seek several opinions until someone says they have Cushing's. No test is 100% accurate. There are several tests that can be used to screen for Cushing's syndrome. If the false positive rate for whatever screening test you perform is 1%, and you test 100 people, then one normal person might be told that he or she has Cushing's wrongly. However we are now testing thousands of normal obese patients, and with a 1% false positive rate, for every 1000 people tested, ten will be told they have Cushing's when they don't. Thus the accuracy of the test changes when you start doing it in large numbers of normal people. It is crucial that we do not remove the pituitary gland of normal people and for this reason it is essential that more tests are carried out to be certain of the diagnosis before operating. Sadly there are a some patients with simple obesity who believe they have Cushing's syndrome and continue to seek opinions until they are told they have Cushing's on one of the tests. Having had some tests that suggest they don't have Cushing's, but one that does, these individuals choose to ignore all the 'normal' tests, and only believe the abnormal one. These individuals do not lose weight when they have had pituitary surgery. It is essential that screening test are not carried out for simple obesity, but that investigations for Cushing's are reserved for patients who have several clinical features of Cushing's such as proximal myopathy which is not present in most patients with simple obesity.

DOI: 10.1530/endoabs.34.N1.1

N1.2

The role of the Endocrine Nurse in the Management of patients with Cushing's syndrome

Wanda Geilvoet
Erasmus MC, Rotterdam, The Netherlands.

Cushing's syndrome is a rare disease and difficult to diagnose. Lots of patients have symptoms many years before diagnosis. A number of different physical and psychological signs and symptoms can be present. After successful surgery, psychological and physical recovery may take a long time. Even after long-term remission of Cushing's syndrome, patients report more negative illness perceptions compared with patients with other acute or chronic conditions. Endocrine nurses can play an important role and can make a difference for these patients during diagnosis, during medical or surgical treatment and in the recovery phase. During the diagnostic phase, nurses need to know why patients must undergo certain endocrine function tests, know how to perform new diagnostic tests, which diagnostic medication needs to be administrated and how to inform the patient. During the operative phase, the endocrine nurse will inform the patient about the pre-operative work up, the surgery and post-operative observations. But it is also important to pay attention to the psychological disturbances patient may suffer from like depression, anxiety and even psychoses. There are some patients who will need a rehabilitation program to work on a better physical condition and to get psychological support during reducing the glucocorticoid replacement therapy to a 'normal level'. Endocrine nurses can make the difference for these patients. In this phase, the endocrine nurse gives education about the recovery process, helps the patient with coping strategies, adherence to medication and self-management. The self-management aspects that come with glucocorticoid replacement therapy, like dose adaptation during medical emergencies, recognising and preventing life threatening situations, like an acute adrenal crisis needs to be highlighted repeatedly.

DOI: 10.1530/endoabs.34.N1.2

N1.3

Why do cured? Cushing's patients need long-term follow-up?

Susan M Webb[1,2,3]
[1]Hospital and IIB SPau, Barcelona, Spain, [2]CIBERER unit 747, ISCIII, Spain, [3]Dept Medicine/Endocrinology, Universitat Autònoma de Barcelona, Spain.

Cushing's syndrome (CS) is associated with systemic complications, which despite successful treatment are not always totally reversible. Morbidity like venous thromboembolism, myocardial infarction, stroke, peptic ulcers, fractures and infections are higher in patients diagnosed with CS, both of adrenal and pituitary origin, and remain elevated during long-term follow-up. In fact, this increased multimorbidity risk is even present in the 3 years before diagnosis, as demonstrated in a large population-based cohort study from Denmark, showing how deleterious chronic hypercortisolism is, despite the apparently benign character of CS.

Treatment of concomitant morbidity like hypertension, insulin resistance and/or diabetes mellitus, dyslipidemia, prothrombotic state, vascular disease, atherosclerosis and increased cardiovascular risk, are often necessary and may not be completely normalized despite disappearance of hypercortisolism after successful treatment. Psychologically, patients complain of impaired memory; objectively loss of brain volume can be seen on CT and MRI scans, as well as cognitive decline and impaired health-related quality of life -HRQoL-, which although is worse in active disease, is still below normative values even years after control of hypercortisolism; together, these limitations contribute to the appearance of depression, a common complaint in these 'cured' patients.

Mortality is higher than in age- and gender-matched subjects, due to complications directly and/or indirectly correlated with glucocorticoid excess. Altogether, despite being a rare disease, difficulties in early diagnosis and residual morbidity represent a significant burden for the patients and for the health system. Thus, the primary goal in the prevention and treatment of complications of CS is correction of hypercortisolism as soon as possible. Furthermore, patients benefit from long-term follow-up, to promptly identify and treat co-morbidities, and the possibility to discuss their experienced limitations with a professional aware that these may not be completely reversible.

DOI: 10.1530/endoabs.34.N1.3

N1.4

Abstract unavailable.

DOI: 10.1530/endoabs.34.N1.4

The journey of endocrine nursing research: from evidence based practice to independent research

N2.1

The journey of endocrine nursing research: from evidence based practice to independent research

Abstract unavailable.

DOI: 10.1530/endoabs.34.N2.1

N2.2

How to audit and evaluate your own practice

Jean Munday
Portsmouth Hospitals NHS Trust, Portsmouth, UK.

Oxford dictionaries define audit as a systematic review or assessment of something.
In their 2002 publication NICE[1] stated that *Clinical audit* is a quality improvement process that seeks to improve patient care and outcomes. Aspects of the structure, processes, and outcomes of care are selected and systematically evaluated against explicit criteria. Where indicated, changes are implemented at an individual, team, or service level and further monitoring is used to confirm improvement in healthcare delivery.
This presentation will use a practical example of an endocrinology audit to demonstrate the process. The aim is to motivate endocrine nurses to undertake their own clinical audits, which will help them evaluate and develop their roles/services.
Reference
1. National Institute for Health and Care Excellence: Principles of Best Practice in Clinical Audit, 2002.
DOI: 10.1530/endoabs.34.N2.2

N2.3

The role of the endocrine nurse in clinical trials

Anne Marland
Oxford University Trust, Oxford, UK.

The objectives of this talk are to outline how the role of the Endocrine nurse fits into clinical trials. Nurses working in clinical trials usually operate at an advanced level, delivering experience, knowledge and autonomous practice. The Endocrine research nurse is responsible for liaising with and co-ordinating the care of Endocrine patients with complex conditions into research studies. Then organising and synchronising these studies by collaborating with other members of the research team and the wider multi-disciplinary team in the management of clinical trials. Attention to the principles of good clinical practice is paramount and also excellent IT skills are required. The ability to organise own workload on a daily basis in accordance with clinical demands is essential.
DOI: 10.1530/endoabs.34.N2.3

N2.4

Independent nursing research

Sofia Llahana
University College Hospital, London, UK.

Research activities such as patient recruitment and data collection for audits and clinical trials led by physicians are common routine practice for a lot of endocrine specialist nurses, however a small percentage of them undertake independent research. Nurses are in a great position of getting research ideas of the ground that can translate back into practice and make a real difference to patient care.
The aim of this presentation is to give practical advice on how to navigate through the process of developing a research question right through to the completion of the study. The following questions will be discussed:

- How to explore research ideas and develop the right research question;
- How to use the PICO(T) strategy to refine your question and select the correct research methodology?
- Qualitative vs quantitative approach, or mixed methods approach?
- Do you need ethics approval?
- How to secure funding for your research study?
- What happens next?

It is important to remember that independent research requires time, a lot of which is actually spent in developing the research question and study design, and ability to work towards deadlines. Although independent, one cannot work in isolation when undertaking a research study and it is vital to discuss your ideas and suggested action points with expert colleagues during every step of the way.
DOI: 10.1530/endoabs.34.N2.4

Young Endocrinologists Session

SfE Young Endocrinologists' prize lectures

YEP1.1

$Sox2^+$ cells of the postnatal pituitary can differentiate into hormone-producing cells *in vivo* and have tumour-inducing potential

Cynthia Andoniadou[1,2] & Juan Pedro Martinez-Barbera[1]
[1]Institute of Child Health, UCL, London, UK; [2]King's College London, London, UK.

The function of stem cells in the anterior pituitary gland, their role in homeostasis during life and possible involvement in pituitary tumours remain largely unknown. We have previously shown that cells exclusively within the $Sox2^+$ compartment of the mouse pituitary have progenitor/stem cell properties in culture where they can self-renew and terminally differentiate. To address if these cells act as stem cells *in vivo*, we generated an inducible *Sox2-CreERT2* mouse line, where we can activate Cre in *Sox2*-expressing cells upon tamoxifen administration. Using genetic lineage tracing, we find that the $Sox2^+$ cell compartment of both the embryonic and adult pituitary contains progenitor/stem cells able to differentiate into all hormone-producing lineages and contribute to organ homeostasis during life. Previously, we demonstrated that the targeted expression of oncogenic β-catenin during embryogenesis, in undifferentiated Rathke's pouch precursors, leads to anterior pituitary tumours resembling adamantinomatous craniopharyngioma. To verify that stem cells are the specific cell type needing to be targeted for tumours to form, we expressed oncogenic β-catenin in adult $Sox2^+$ cells using our inducible model. Confirming this notion, we find that pituitary tumours do form, but unexpectedly, the tumour mass does not carry the oncogenic mutation; genetic lineage tracing reveals that the mutation-sustaining $Sox2^+$ stem cells are not the cell-of-origin of the tumours. Investigating the mechanism of tumorigenesis, we demonstrate that $Sox2^+$ cells targeted with mutant β-catenin express a host of secreted signalling factors, capable of stimulating proliferation and transformation of surrounding cells in a paracrine fashion. In summary, our data demonstrate that $Sox2^+$ cells are progenitor/stem cells *in vivo*, and in addition, we have revealed a novel paracrine mechanism implicating $Sox2^+$ cells as instigators of pituitary oncogenesis. Our results have profound implications in the understanding of craniopharyngioma and other pituitary tumours such as adenoma and could facilitate the development of new therapies.

DOI: 10.1530/endoabs.34.YEP1.1

YEP1.2

Clinical effects of kisspeptin on reproductive hormone secretion, LH pulsatility and oocyte maturation

Channa Jayasena, Ali Abbara, Alexander Comninos, Gurjinder Nijher, Johannes Veldhuis, Anna Carby, Geoffrey Trew, Mohammad Ghatei, Stephen Bloom & Waljit Dhillo
Imperial College London, London, UK.

A decade ago, genetic disruption of the kisspeptin signalling pathway was observed to cause hypogonadotrophic hypogonadism. Hypothalamic kisspeptin acts by stimulating secretion of endogenous GnRH. Our research has investigated the physiological effects of kisspeptin in women, with the overarching aim of determining if kisspeptin could be used to treat women with infertility.

We initially observed that s.c. bolus injection of kisspeptin acutely stimulated gonadotrophin secretion in healthy women, and patients with functional hypothalamic amenorrhoea due to weight loss or exercise (FHA). Furthermore, the mean amplitude of LH pulses was increased by kisspeptin in healthy women and patients with FHA. By contrast, we observed that exogenous neurokinin B (co-expressed with kisspeptin) does not alter sex hormone secretion in healthy women. We also investigated the clinical effects of chronic kisspeptin administration. In healthy women, twice-daily kisspeptin advanced the menstrual cycle by 2 days. In patients with FHA, 8 h i.v. infusion of kisspeptin increased only basal LH secretion at low doses, increased basal and pulsatile LH secretion at intermediate doses, and caused tachyphylaxis at the highest dose. Like GnRH, kisspeptin may therefore have distinct and paradoxical effects on the human reproductive axis. Kisspeptin is needed for ovulation in multiple species. We therefore conducted a phase 2 trial determining if a single injection of kisspeptin induces oocyte maturation during IVF treatment. Oocyte maturation and fertilisation was observed in 45/47 and 43/47 of patients, respectively. Clinical pregnancy has been confirmed in 14/44 patients; two patients have already given birth to healthy babies.

Our human studies reveal that kisspeptin potently stimulates gonadotropin secretion, and accentuates LH pulsatility in women. Furthermore kisspeptin can induce oocyte maturation in women undergoing IVF treatment. Further studies are underway to determine if kisspeptin offers a therapeutic advantage over existing treatments for infertility.

DOI: 10.1530/endoabs.34.YEP1.2

Establishing successful collaborations

YE1.1

Abstract unavailable

DOI: 10.1530/endoabs.34.YE1.1

YE1.2

How do I talk to a bioinformatician/mathematician/engineer?

Craig McArdle
School of Clinical Sciences, University of Bristol, Bristol, UK.

As we learn more and more about the complexity of biological systems there is a real risk of being drowned in data but starved of information. This is increasingly recognised by grant funders and is certainly true of the signal transduction field where the complexity of signaling networks makes our traditional intuitive approaches look increasingly inadequate. For my own work on GnRH signaling, one solution has been to collaborate with mathematicians and engineers. Success in such collaboration requires not just shared interests but also establishment of shared language and shared understanding of what can and can't be done. Such collaboration brings different perspectives and skill-sets to bear on biological questions. In this session, I'll discuss what we've learnt about how to exploit (and not be undermined by) these differences.

DOI: 10.1530/endoabs.34.YE1.2

YE1.3

Abstract unavailable

DOI: 10.1530/endoabs.34.YE1.3

YE1.4

Biobanking: what is it, and how could it be beneficial to me?

Jon Tobias
Musculoskeletal Research Unit, Bristol, UK.

A biobank is a repository that stores biological samples (usually human) for research. Samples comprise a range of tissues, of which the most common are blood samples. The latter are often separated into different constituents such as serum, DNA and RNA extracts at the time of initial collection. Cell lines can also be generated, thereby perpetuating material for DNA and related analyses. Generally, biological samples are stored in biobanks as part of a protocol pertaining to a defined population. The latter may comprise a representative sub-sample of the wider population, for example as part of a population based cohort.

Alternatively, participants may be defined by a particular disease. Biological samples in biobanks are generally linked to other information about the individual, collected systematically as part of the same overall process. This may consist of a core dataset comprising clinical information collected in clinics for patient cohorts, or in the case of population based cohorts, information obtained from research clinics to which patients are invited or subsequent questionnaires. It is also possible to gain increasingly detailed clinical information through linkage to other databases such as HES.

Biobanks represent an important resource for research. As well as enabling the study of relationships between factors analysed in stored tissues and disease processes, the fact that information about phenotypes and exposures is collected in a standardised way may provide an important resource for subsidiary studies not involving the actual samples. Moreover, assuming data is collected in a standardised way, it may be possible to combine information with other similar biobanks, which may be essential for achieving sufficient power to investigate small effects such as those observed in genome wide association studies. Examples of research findings obtained from biobanks will be discussed using the Avon Longitudinal Study of Parents and Children (ALSPAC) as an example of a population based cohort, and the High Bone Mass study as an example of a cohort defined by a particular disease/characteristic.

DOI: 10.1530/endoabs.34.YE1.4

YE1.5

Peer-to-peer collaborations: importance of YE groups

Karen Chapman
University of Edinburgh, Edinburgh, UK.

The value of mentoring by an experienced, usually older colleague, is widely acknowledged, but just as important is the value of sharing experiences, knowledge and support within a peer group. Peer-to-peer collaboration, or peer mentoring, provides opportunities for networking, friendship, education and career development and advice. Formal groupings and networks often exist locally in the form of student or postdoc societies. The Young Endocrinologists of the Society for Endocrinology provide a national network. By working together, they form a lobby group promoting the interests of early career researchers. This is effective at many levels, not least in representing our early career researchers at parliamentary events over the last couple of years. Within the Society for Endocrinology, the YEs have demonstrated that they can shape Society policy to promote their needs and interests, as well as providing a valuable and lasting network of friendships and collaborations to those at the outset of their career.

DOI: 10.1530/endoabs.34.YE1.5

Senior Endocrinologists Session

SE1.1

How important is luck for major scientific discoveries?

Jamshed Tata
Senior Endocrinologist, London, UK.

It is increasingly common for research funding bodies to ask applicants to demonstrate how the outcome of the proposed research will benefit society or the economic wellbeing of the country. This pre-supposes that the results of the research are somehow predictable and thus diminishes the importance of 'blue skies' or curiosity-driven research and the role played by luck or chance in major scientific discoveries. I shall describe how throughout history accidental findings, 'hunches' and chance encounters have led to some major discoveries. Whether or not the over-flowing bath of Archimedes or Newton's apple falling to ground are simply anecdotal, they are linked to dramatic changes in so many branches of physics and led to numerous applications in engineering technologies. More recently, the contamination of Alexander Fleming's bacteriological plates by fungal spores from a neighbouring laboratory leading to the development of antibiotics is a good example of the value of accidents leading to major advances. 'Hunches' or 'dreams' have also been cited as the seeds of great scientific advances. August Kekulé's dream of a snake biting its own tail has been credited with the birth of the benzene ring and that of a major new branch of organic chemistry, without which the development of chemical dyestuffs and plastics would not have been possible. The mistake by Hideki Shirakawa's student and his chance encounter with Alan MacDiarmid led to the development of conducting polymers in present-day electronics. Finally, it is well to consider Louis Pasteur's statement that chance only favours the prepared minds, important roles of 'blind alleys' and 'wrong ideas', stressed by Peter Medawar and Richard Feynman, which are impossible to explain in a grant application.

DOI: 10.1530/endoabs.34.SE1.1

SE1.2

1776: revolution in liverpool: Matthew Dobson discovers hyperglycaemia

Ian Macfarlane
University Hospital Aintree, Liverpool, UK.

Mattew Dobson (1735–1784) was a Liverpool physician who was recognised with FRS for his numerous and varied publications. He investigated a patient with diabetes, which at the time was considered to be a kidney disorder, associated with excessive sweet tasting urine. His experiments showed that the sweet urine, on evaporation, contained white granular material indistinguishable from sugar. However, he also made the crucial observation that the blood serum was also sweet to taste. He concluded that the emaciation in diabetes was due to 'a large proportion of the alimentary matter being drawn off by the kidney before assimilation'.

This revolutionary discovery sent diabetes research in the correct direction studying the mechanisms by which the body deals with carbohydrate foods and appropriate treatments followed. It was published in 1776 in Medical Observations and Enquiries… a journal published by a group of physicians who met alternate monday evenings in the Mitre Tavern, Fleet Street, London. At the time another revolution was taking place across 'The Pond'!

DOI: 10.1530/endoabs.34.SE1.2

SE1.3

Macroprolactin, to seek or ignore: a trans-Atlantic division

T Joseph McKenna
St Vincent's University Hospital, Elm Park, Co Dublin, Ireland.

Macroprolactin is a bio-inactive molecule which cross-reacts in prolactin immune-assays. 'Macroprolactinaemia' describes hyperprolactinaemia which is entirely explained by the presence of macroprolactin. Failure to recognize macroprolactinaemia is associated with inappropriate investigation and unnecessary treatment. While screening for macroprolactinaemia is regularly undertaken in the UK and Ireland and to a lesser extent in continental European and Japan, this is rarely performed in the USA. Based on information supplied by the College of American Pathologists it is estimated that 5–10% of hyperprolactinaemia in the USA is due to macroprolactinaemia. The Endocrine Society guideline for 'Diagnosis and treatment of hyperprolactinaemia' recognizes that 45% of macroprolactinaemic patients have oligo-/amenorrhoea. However, the guideline recommends that screening for macroprolactin should be limited to 'asymptomatic hyperprolactinaemic subjects'! (Melmed *et al*. *J Clin Endocrinol Metab* 2011 **96** 273–288). Prompted by this recommendation a letter to the Editor from an international group of experts on macroprolactin was published on line (Fahie-Wilson *et al*. Published 14 Jan 2013). The letter requests that the guideline dealing with macroprolactin be revised to conform with published evidence. A recent review article (Klibansky. Prolactinomas. *N Engl J Med* 2010 **362** 1219–1226) noted macroprolactinaemia but concluded that 'such occurrences are rare'. The author cited Gibney *et al*. 2005, to support this claim although this publication reports that 22% of hyperprolactinaemic patients had macroprolactinaemia! (The impact on clinical practice of routine screening for macroprolactin. *J Clin Endocrinol Metab* **90** 3927–3932). A letter to the Editor indicating this error was not accepted for publication, but subsequently under 'corrections' it was noted the phrase 'such occurrences are rare' should not have been included (*N Engl J Med* 2010 **362** 2142).

This presentation will explore why these differences in trans-Atlantic medical practices exist and discuss potential approaches to bring about change to improve patient care.

DOI: 10.1530/endoabs.34.SE1.3

SE1.4

The Foundling hospital

Brian Cooke
2 Prospect Lane, Harpenden, UK.

In 1739 Thomas Coram was granted a Royal Charter to establish the Foundling hospital for the care of abandoned babies. The hospital was established in Bloomsbury and had the patronage of leading cultural figures of the day, including William Hogarth and Handel. Babies were admitted by ballot (white ball in, red ball maybe and black ball rejected), given a new name and fostered until the age of 5. Upon admittance to the hospital they severed all contact with their foster parents. They were given a uniform and subjected to a rigorous regime. Their education was basic. Girls were prepared mainly for domestic service and the boys for the military. Music, religion and exercise were important. The children had access to first class medical expertise.

The regime adopted by the Foundling hospital raises questions about its effects on the emotional stability of the Foundlings and their preparation for adult life but it also warrants a comparison with our failures in contemporary care homes.

DOI: 10.1530/endoabs.34.SE1.4

SE1.5

Steroid merchants of Edinburgh

J Ian Mason[1,2]
[1]MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, Scotland, UK; [2]Biochemistry Department, University of Buffalo, Buffalo, New York, USA.

We present a commentary based on both history and experiences of the many contributions to steroid biochemistry and endocrinology made in Edinburgh over the past 75 years. Reflections will be made commencing with the contributions of Guy Marrian (1904–1981) and colleagues. Marian, occupied the Chair of Medicine with relation to Medicine (later Medical Biochemistry, then Biochemistry) at Edinburgh University from 1939 until his appointment as Director of the ICRF Laboratories at Lincoln Inns Field in 1958. Two members of his Edinburgh Department, Jim Grant (1916–2004) and George Boyd (1924–1983) continued to make important contributions to steroid biochemistry in Scotland, both of whom were mentors to young investigators who subsequently gained international reputations. In 1983, Christopher Edwards was appointed Professor of Medicine at the Western General Hospital that led to another family of internationally recognised steroid exponents. Mention will also include contributions made by the numerous steroid experts of the MRC Clinical Endocrinology Research Unit (1946–1971), the brainchild of Marrian, John Gaddum and others. The initial scientists included Barbara Clayton, Jim Brown, Arnold Klopper and John Loraine. After its closure the MRC Reproductive Biology (latterly Human Reproductive Sciences) Unit (1972–2011) was opened with Roger Short as Director and included David Baird, Professor of Obstetrics and Gynaecology. Subsequent Directors were Dennis Lincoln and Robert Millar. In 2011, this unit evolved formally into the MRC Centre for Reproductive Health (Jeffrey Pollard, Director) within the University of Edinburgh.

DOI: 10.1530/endoabs.34.SE1.5

SE1.6

Reproductive biology in the enlightenment: some contributions of the hunter brothers

Brian Cook
Royal Infirmary, Glasgow, UK.

In 1807, the Hunterian museum, the first public museum in Scotland, opened at the University of Glasgow. Its contents were bequeathed by William Hunter, a graduate of the university, and included, as well as anatomy specimens, books, coins, paintings, natural history specimens, 'curiosities from the South Seas', minerals, fossils and so forth. A letter of 1809 stated 'Hunter's museum has been here for some time and drawn a considerable number of students to this place. Some years ago there were not a hundred medical students in Glasgow now there is more than double that number'. The specimens were used by William Hunter in his anatomy school in London. He was the greatest anatomy teacher of his day and his reputation as a lecturer was so substantial that men such as Adam Smith, Edward Gibbon and Edmund Burke went to hear him. John, William's brother, initially worked as his assistant but later also developed a museum of specimens of natural history and human anatomy which has passed into the care of the Royal College of Surgeons in London. Both men were gifted scientific investigators, each maintained a successful medical practice and each developed remarkable collections. Their contributions to reproductive biology include William's great treatise, 'The Anatomy of the Human Gravid Uterus', the discovery of the decidual reaction, the elucidation of the relationship between follicles, ova and corpora lutea, the descent of the testes, the nature of the free-martin and other 'hermaphrodites', and the use of artificial insemination. The scope of their work was tremendous and reflects the ferment of the times when Scotland was the intellectual centre of Europe, a time which we now know as the enlightenment.

DOI: 10.1530/endoabs.34.SE1.6

Oral Communications

Young Endocrinologists prize session

OC1.1

The Thr92Ala substitution in deiodonase-2 is associated with increased odds of a sub-optimal IQ score in children with low-normal thyroid function

Peter Taylor[1,2], Onyebuchi Okosieme[1], Adrian Sayers[2], Kate Northstone[2], Mohd Draman[1], Kirsty Stevenson[3], Wolf Woltersdorf[3], Andrew Taylor[4], Elizabeth Pearce[5], Vijay Panicker[6], Marian Ludgate[1], John Gregory[1], John Lazarus[1], Nicholas Timpson[2], Sue Channon[6] & Colin Dayan[1]
[1]Cardiff University, Cardiff, UK; [2]University of Bristol, Bristol, UK; [3]Bristol Royal Infirmary University Hospitals Bristol NHS Foundation Trust, Bristol, UK; [4]Royal United Hospital, Bath, UK; [5]Boston University, Boston, Massachusetts, USA; [6]St David's Children's Centre, Cardiff, UK.

Importance
Thyroid hormone is essential for cognitive development. The Thr92Ala substitution in deiodonase-2 appears to reduce intracellular availability of active thyroid hormone. Individuals with low-normal serum thyroid hormone levels and this substitution might have insufficient intracellular thyroid hormone for optimal cognitive development.
Objective
To explore whether individuals with low thyroid hormone bioavailability – free thyroxine in the lowest quartile and homozygous for the Thr92Ala substitution in deiodonase-2 – had higher odds of an IQ <85.
Design
Population based cohort study
Participants and setting
3123 individuals with genetic data, thyroid function (at age 7) and cognitive assessments (at age 8) available in a population-based birth cohort, the Avon Longitudinal Study of Parents and Children.
Main outcome measures
The odds of having an IQ score ≤ 85 in children with low thyroid hormone bioavailability compared to the rest of the study population. Analyses were adjusted for age, gender, ethnicity, thyroid hormone parameters, markers of social class and early life environment.
Results
Children with low thyroid hormone bioavailability had higher odds of a total IQ score <85 (OR $=4.15$, 95% CI 1.60, 10.8, $P=0.003$) and a lower mean IQ ($P=0.003$) compared to individuals with free thyroxine above the lowest quartile not homozygous for the Thr92Ala substitution. There was no evidence of association with IQ in individuals with free thyroxine in the lowest quartile, or with the Thr92Ala substitution alone; however we observed evidence of interaction between free thyroxine in the lowest quartile and the Thr92Ala substitution in their relationship with IQ ($P=0.008$).
Conclusions
Common genetic variation in the intracellular thyroid hormone pathway appears to substantially modify the effect of low-normal serum thyroid hormone levels on IQ. This highlights the importance of the intracellular pathway in determining an individual's thyroid status and raises the possibility that levothyroxine supplementation in a genotype dependent manner to children with lower thyroid hormone levels may improve cognitive outcomes.

DOI: 10.1530/endoabs.34.OC1.1

OC1.2

Characterisation of adrenocortical progenitor cells: isolation of mesenchymal stem cell-like cell populations from human adrenal cortex

Earn Gan[1,2], Wendy Robson[2], Thomas Lennard[1,2], Simon Pearce[1,2] & Rachel Oldershaw[1]
[1]Newcastle University, Newcastle upon Tyne, UK; [2]Royal Victoria Infirmary, Newcastle upon Tyne, UK.

Background
The plasticity of adrenal cortex suggests the presence of adrenocortical stem cells (ACSC) but the exact *in vivo* identity of ACSC remains elusive. A few studies have demonstrated the differentiation of adipose or bone marrow-derived mesenchymal stem cells (MSC) into steroid-producing cells. We therefore investigated the direct isolation of MSC from human adrenal cortex.
Methods
Adrenals were obtained as discarded surgical material. Single cell suspensions from human adrenal cortex ($n=3$) were cultured onto either complete growth medium (CM) or mesenchymal stem cell growth promotion medium (MGPM). Following *ex-vivo* expansion, the adipogenic, chondrogenic and osteogenic differentiation potential of these cells were evaluated. Cells were analysed by flow cytometry for cell-surface antigens associated with bone marrow-MSCs and adrenocortical-specific phenotype. Multipotent differentiation was assessed by immunocytochemistry.
Results
The formation of colony forming unit-fibroblasts consisting of adherent cells with fibroblast morphology were observed from the monolayer cell culture of human adrenal cortex in both CM and MGPM. Cells derived and cultured in MGPM revealed differentiation toward osteogenic and adipogenic cell lineages as determined by positive staining for Alizarin Red and Oil Red-O, respectively. Chondrogenic staining could not be demonstrated. These cells expressed cell-surface MSC markers (CDs: 44, 90, 105, 166) but did not express the haematopoietic and lymphocytic markers (CD19 and CD45). The proportion of MSC-positive cells was lower in CM compared to those from MGPM (mean 90.5 vs 70.2%; $P=0.001$). These cells also demonstrated higher expression of GLI1 ($P=0.035$) and DAX1 ($P=0.009$) antigens. More than 90% of the cells in MGPM were shown to co-express SF1 and MSC markers. They were highly proliferative as judged by GLI/SHH expression.
Conclusion
Our study demonstrates, for the first time, that cells derived from human adrenal cortex cells have the capacity to differentiate into mesenchymal lineages. Understanding the cell biology of adrenal-cortex derived MSCs will inform regenerative medicine approaches in Addison's disease.

DOI: 10.1530/endoabs.34.OC1.2

OC1.3

Angiogenic factors sFlt1 and PlGF are novel determinants of newborn thyroid (dys)function: the Generation R Study

Tim Korevaar, Sarah Schalekamp-Timmermans, Theo Visser, Yolanda de Rijke, Edward Visser, Willy Visser, Sabine de Muinck Keizer-Schrama, Albert Hofman, Herbert Hooijkaas, Henning Tiemeier, Jacoba Bongers-Schokking, Vincent Jaddoe, Eric Steegers, Marco Medici & Robin Peeters
Erasmus University Medical Center, Rotterdam, The Netherlands.

Introduction
Adequate thyroid hormone availability during early life is crucial for normal child growth and development. Fetal growth *in utero* heavily depends on angiogenesis. Placental growth factor (PlGF) is a proangiogenic factor sharing high homology with vascular endothelial growth factor (VEGF) whereas soluble FMS-like tyrosine kinase-1 (sFlt1) is a potent antagonist of VEGF and PlGF signaling. Since the thyroid is a highly vascularized organ, we hypothesized that fetal angiogenic factors influence *in utero* thyreogenesis and therefore investigated the effects of sFlt1 and PlGF on newborn thyroid (dys)function.
Methods
sFlt1, PlGF, TSH and free T_4 were determined in cord blood of 3525 newborns from the Generation R Cohort. Analyses were adjusted for relevant maternal and child covariates.
Results
sFlt1 levels were positively associated with TSH (β (S.E.M.) $+0.07$ (0.02); $P=0.0006$) and inversely with FT_4 (β (S.E.M.) -0.58 (0.11); $P<0.0001$). PlGF showed a positive association with FT_4 (β (S.E.M.) $+0.19$ (0.02); $P<0.0001$). Elevated levels of sFlt1 were associated with a 3.2-fold increased risk of hypothyroxinemia ($P=0.02$). Decreased levels of PlGF were associated with a 7.3-fold increased risk of hypothyroxinemia ($P<0.001$). Within the normal range, a dose dependent effect of sFlt1 on thyroid dysfunction was observed: high-normal sFlt1 levels were associated with a 15.3-fold increased risk of hypothyroxinemia and a 2.9-fold increased risk of hyperthyrotropinemia (both $P<0.0001$).
Conclusion
Angiogenic factors sFlt1 and PlGF are novel determinants of newborn thyroid function. Most likely, this is mediated through effects on *in utero* thyreogenesis. Abnormal as well as normal-range sFlt1 and PlGF levels influence the risk of newborn thyroid dysfunction which has been associated with adverse neurodevelopmental effects. These data provide important novel insights into the physiology of thyreogenesis and into the etiology of newborn thyroid (dys) function.

DOI: 10.1530/endoabs.34.OC1.3

OC1.4

Single molecule analysis of GPCR transactivation reveals oligomeric complexes that regulate signal sensitivity

Kim Jonas[1], Francesca Fanelli[2], Ilpo Huhtaniemi[1,3] & Aylin Hanyaloglu[1]
[1]Imperial College London, London, UK; [2]University of Modena, Modena, Italy; [3]University of Turku, Turku, Finland.

G protein-coupled receptors (GPCRs) are the largest family of mammalian receptors, modulating most aspects of endocrine homeostasis. GPCRs can associate to form dimers and higher order oligomers, acting to diversify receptor functionality. Most studies have explored the functional significance of GPCR dimerization *in vitro* and we have recently demonstrated the functional relevance of GPCR dimerization *in vivo*. Using the LH receptor (LHR), we have shown that targeted co-expression of ligand-binding deficient (LHR^{B-}), and signalling-defective (LHR^{S-}) LHR restored the gonadal function and fertility of male LHR knockout mice, showing that dimerisation is a physiologically relevant form of GPCR-mediated signalling. As transactivation is a complex form of signal regulation, we went on to study the molecular aspects of GPCR di/oligomerisation *in vitro*, utilising these LHR^{B-} and LHR^{S-} transactivating mutants. Using the single molecule imaging technique of photo-activated localisation microscopy (PALM), we have probed the molecular composition of both WT LHR, and transactivating $LHR^{B-/S-}$ complexes, with a localisation precision of $\sim$20 nm. Both WT and transactivating LHR complexes formed dimers and higher order oligomeric complexes. Surprisingly, the transactivating mutant $LHR^{B-/S-}$ appeared to favour hetero-oligomeric ($LHR^{B-/S-}$) complexes, therefore we determined if this difference in association impacted on ligand-dependent G-protein pathways using human chorionic gonadotrophin (hCG) and LH. Both LH and hCG produced comparable Gs-dependent responses in WT LHR and $LHR^{B-/S}$, however, LH-dependent Gq pathways were attenuated in the transactivating model. No differences were observed in associated forms in either WT or $LHR^{B-/S-}$ complexes. Structural modelling and spatial arrangement analysis via PALM provided insight into how the transactivating mutant receptors associate into dimeric and higher order complexes. These studies show us how di/oligomerization may modulate the functionality of one receptor subtype, providing key insights into important mechanisms of how di/oligomerisation impacts on the physiology of GPCRs in endocrine systems.

DOI: 10.1530/endoabs.34.OC1.4

OC1.5

Kisspeptin: a novel physiological trigger for oocyte maturation in IVF treatment

Ali Abbara[1], Channa Jayasena[1], Alexander Comninos[1], Monica Nijher[1], Georgios Christopoulos[2], Chioma Izzi-Engbeaya[1], Mathini Sridharan[1], Shankunthala Narayanaswamy, Deborah Ashby[3], Mohammad Ghatei[1], Steve Bloom[1], Anna Carby[2], Geoffrey Trew[2] & Waljit Dhillo[1]
[1]Section of Investigative Medicine, Imperial College London, London, UK; [2]Hammersmith IVF Unit, Imperial College NHS Trust, London, UK; [3]Clinical Trials Unit, Imperial College London, London, UK.

Background
IVF is an effective treatment for infertility, but can cause the ovarian hyperstimulation syndrome (OHSS), which is associated with serous effusions, cardiac tamponade, circulatory collapse, and even death. Injection of human chorionic gonadotrophin (hCG) stimulates oocyte maturation during IVF, but is also the major cause of OHSS. The hypothalamic neuropeptide kisspeptin, stimulates endogenous LH secretion in a GnRH-dependent manner. A more physiological stimulus for oocyte maturation may avoid OHSS and thereby improve the safety of IVF treatment.
Aim
To determine if kisspeptin can induce oocyte maturation during IVF treatment.
Methods
Women underwent a modified recombinant FSH/GnRH antagonist IVF protocol, administering kisspeptin instead of hCG to trigger oocyte maturation. Daily recombinant FSH treatment commenced from menstrual day 2. GnRH-antagonist treatment was used to inhibit a premature LH surge. Once three follicles grew to $\geq$18 mm in diameter, a single s.c. bolus injection of kisspeptin (1.6–3.2 nmol/kg, $n=5$; 6.4 nmol/kg, $n=21$; 12.8 nmol/kg, $n=21$) was administered 24 h after the final GnRH-antagonist injection. Oocytes were retrieved 36 h after kisspeptin injection. Following ICSI, one to two embryos were transferred to the uterine cavity. Primary outcome was the number of mature oocytes (oocytes in metaphase II; MII).
Results
Forty-seven women completed the study. Kisspeptin increased serum LH 9.0$\pm$7.5-fold (mean$\pm$S.D.) 12 h following injection. Oocyte maturation was observed at all doses of kisspeptin: 96% (45/47) of women had oocyte maturation, with 7.9$\pm$4.1 MII oocytes/cycle. Fertilisation occurred in 91% (43/47) cases, with 5.7$\pm$3.5 zygotes/cycle. To date, clinical pregnancy has been confirmed on ultrasound at 6 weeks gestation in 10/44 (23%) patients receiving kisspeptin; two of these patients have already given birth to healthy baby boys.
Conclusion
We show for the first time that kisspeptin can effectively induce oocyte maturation in IVF treatment. Kisspeptin may therefore offer an entirely novel and potentially safer therapeutic option for fertility treatment.

DOI: 10.1530/endoabs.34.OC1.5

OC1.6

A loss-of-function mutation in the prolactin receptor causes familial hyperprolactinaemia

Caroline Gorvin[1], Paul Newey[1], Stephen Cleland[2], Christian Willberg[3], Marcus Bridge[4], Mohammed Azharuddin[5], Russell Drummond[5], Anton van der Merwe[4], Paul Klenerman[3], Chas Bountra[6] & Rajesh Thakker[1]
[1]Academic Endocrine Unit, Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK; [2]Hampshire Hospitals Foundation Trust, Hampshire, UK; [3]Peter Medawar Building for Pathogen Research, Nuffield Department of Medicine, University of Oxford, Oxford, UK; [4]Sir William Dunn School of Pathology, Oxford Molecular Pathology Institute, University of Oxford, Oxford, UK; [5]Glasgow Royal Infirmary, Glasgow, UK; [6]Structural Genomics Consortium, University of Oxford, Oxford, UK.

The prolactin receptor (PRLR) is a member of the class I cytokine receptor family that signals predominantly through the JAK2–STAT5 pathway. To date, PRLR mutations have not been established to be associated with any disorders. Here, we report a PRLR mutation (His188Arg) that caused familial hyperprolactinaemia in three sisters, two of whom presented with oligomenorrhea and one with infertility. The hyperprolactinaemia was not associated with pituitary tumours, which were sought for using MRI, or mutations of the multiple endocrine neoplasia type I (*MEN1*) or aryl-hydrocarbon-interacting protein (*AIP*) genes. We therefore hypothesised that the familial hyperprolactinaemia may be due to either abnormalities of the prolactin gene, with secretion of biologically inactive forms of prolactin, or prolactin insensitivity due to a *PRLR* mutation. DNA sequence analysis of leukocyte DNA did not identify any prolactin gene abnormalities, but revealed a heterozygous missense (His188Arg) mutation, involving an evolutionarily conserved residue in all three hyperprolactinaemic sisters. The functional effects of this mutation were investigated by expression in HEK293 cells and use of phosphorylation and STAT5 amplified luminescence proximity homogeneous (AlphaScreen) assays, as well as a STAT5-dependent gene expression assay that utilised a cytokine-inducible Src homology 2 domain containing protein (CISH) luciferase reporter. The PRLR Arg188 mutant abolished phosphorylation of JAK2 and STAT5 proteins, and STAT5 phosphorylation was demonstrated to be absent at all time-points (0–60 min) and prolactin concentrations (0–1000 ng/ml) when compared to the WT His188 PRLR. In addition, the PRLR Arg188 mutant abolished CISH reporter expression when compared to WT. Moreover, co-transfection of WT and mutant constructs also resulted in a reduction of CISH reporter expression, consistent with a loss-of-function, and a likely dominant-negative action of the mutant PRLR. Thus, our studies have identified that a loss-of-function PRLR germline mutation that results in prolactin insensitivity is a cause of familial hyperprolactinaemia.

DOI: 10.1530/endoabs.34.OC1.6

Endocrine regulation of cell behaviour

OC2.1

A novel modulator of cell invasion and metastasis

Rachel Watkins, Vicki Smith, Neil Sharna, Martin Read, Perkin Kwan, Rachel Fletcher, Kristien Boelaert, Jayne Franklyn & Chris McCabe
University of Birmingham, Birmingham, UK.

Metastasis is a multistep process responsible for the vast majority of endocrine cancer deaths. We have previously identified the proto-oncogene PBF to be upregulated in differentiated thyroid cancer, and recently PBF expression has been correlated with distant thyroid cancer metastasis at diagnosis. Further, PBF potently induces breast cancer cell invasion *in vitro*, and our recent *in vivo* data demonstrate that colorectal tumours with higher PBF protein expression demonstrate increased vascular invasion. We now show that PBF significantly promotes cell invasion in thyroid carcinoma SW1736 cells compared to vector only (VO) ($P<0.01$). Cell invasion is related to and encompasses cell migration.

In keeping with PBF's role in cell invasion, overexpression of PBF in HCT116 cells induced ~60% greater migration compared to VO-transfected controls ($P<0.001$), implying a role for PBF in promoting cell movement. These data were supported by wound healing assays in MDA-MB-231 cells, which revealed that GFP-tagged PBF cells migrate significantly further than GFP-VO cells (VO = 115.3 μm, PBF = 143.0 μm, $P<0.01$). To begin to unravel the mechanism by which PBF promotes cell migration and invasion we used an IP-MS approach to discover PBF binding partners, and identified the cortical actin binding protein, cortactin. Interaction was confirmed through co-IP, immunfluorescence and proximity ligation assays. The latter additionally indicated that this interaction occurs within or close to the plasma membrane. PBF is phosphorylated by SRC at tyrosine 174 (Y174) and when phosphorylated it is preferentially located at the plasma membrane. PLA assays using the SRC inhibitor PP1 and co-IP experiments using a PBF phospho null mutant (Y174A) demonstrated that loss of PBF phosphorylation at Y174 abrogates the interaction between PBF and cortactin, suggesting that cortactin preferentially binds to phosphorylated PBF. We therefore examined the ability of the phosphorylation status of PBF to modulate its induction of cell invasion. Substitution of tyrosine 174 resulted in a total loss of invasive capacity, confirming the critical importance of this residue. Taken together these data suggest that PBF induces cell invasion and migration across a range of cell lines, and that this occurs via its interaction with cortactin.

DOI: 10.1530/endoabs.34.OC2.1

OC2.2

Inhibition of human NET cell proliferation by a peptide identified through phage display screening

Mark Stevenson[1], Kate Lines[1], Lykourgos-Panagiotis Zalmas[1], Mahsa Javid[1], Juris Galvanovskis[1], Simona Grozinsky-Glasberg[2], Matthew Wood[1], Ashley Grossman[1] & Rajesh Thakker[1]
[1]University of Oxford, Oxford, UK; [2]Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

Neuroendocrine tumours (NETs) occur in multiple sites including, the pancreas, gastrointestinal tract, lung, thymus, parathyroid, adrenals and pituitary. Current treatments for advanced NETs such as surgery, chemotherapy or radiotherapy, rarely achieve a cure due to metastases at presentation therefore additional therapeutic treatments are required. Identification of cell surface receptors or binding sites that are unique or up-regulated on tumour or neuroendocrine tissue could lead to novel targeted drugs, radio-isotope or gene therapy treatments. To identify ligands to such receptors we used a phage display selection technique in which a library of peptides is expressed on the outside of phage virions, with each virion displaying a unique peptide encoded by the genetic material inside. Three rounds of phage display screening using a 12-mer library (New England BioLabs) to three human neuroendocrine (carcinoid) cell lines, BON-1, H727 and H720, which were shown to express chromogranin A, neuron specific enolase and menin, was performed and binding phage identified. A lead candidate peptide ALWPPNLHAWVP emerged since it was recovered in 81% of BON-1 cell binding clones and 73% of H727 cell binding clones, with the frequency of ALWPPNLHAWVP-bearing phage increasing from the second to the third round, confirming that this peptide was enriched during screening. Incubation of BON-1 cells with biotinylated ALWPPNLHAWVP peptide or a scrambled peptide control followed by FITC–avidin and confocal microscopic analysis confirmed the ability of the peptide to bind to these cells. Application of 10 μM ALWPPNLHAWVP mediated a 70% decrease ($P<0.005$) in BON-1 and 35% decrease ($P<0.05$) in H727 cell proliferation after 6 days, compared to the scrambled control peptide. This was in part mediated by a 1.23-fold ($P<0.02$) increase in apoptosis, without an increase in cell senescence. Thus, our studies have identified a peptide that may play a role in targeting NET cells and in reducing NET tumour growth.

DOI: 10.1530/endoabs.34.OC2.2

OC2.3

A20 confers protection against TRAIL-induced apoptosis in the pancreatic β-cell

Rebecca Yates[1], Jonathan Robinson[1], Buthainah Al-Azzawi[1] & Catriona Kelly[2]
[1]Keele University, Stoke-on-Trent, UK; [2]University of Ulster, Londonderry, UK.

Abstract

Background

β-cell apoptosis is a cardinal feature of type 1 diabetes. A20 is an important negative regulator of inflammation and apoptosis and protects against β-cell death in response to inflammatory cytokines, and β-cell rejection following islet transplantation[1]. Preliminary experiments showed rapid induction of A20 (15–60 min) in β-cells in response to treatment with TNFα. Experimental data suggests that TNF-related apoptosis-inducing ligand (TRAIL), an immune system modulator protein, is important in the pathogenesis of type 1 diabetes[2]. Recent evidence from glioblastoma cells now suggests that A20 regulates TRAIL-mediated apoptosis through inhibition of caspase-8[3]. However, this relationship has not been examined in the β-cell.

Methods

The BRIN–BD11 β-cell line was used for all experiments, which included untreated cells as a control group, cells treated with a commercially available A20 siRNA, and cells treated with recombinant A20 (100 ng/ml). A20 knockdown was achieved using siRNA against A20 (Qiagen). All cells were subsequently treated with recombinant TRAIL (100 ng/ml) for 0, 1, 2, 4 and 24 h. The induction of A20 and cleaved caspase-8 was assessed over time by qPCR, while the effect on cellular viability was measured by MTT assay.

Results

We confirmed the induction of A20 in response to TNFα in BRIN–BD11 cells initially, and subsequently showed that TRAIL enhanced A20 mRNA expression in a similar manner. Peak TRAIL-induced A20 expression was observed after 1 h ($P<0.01$). In A20-silenced cells, TRAIL treatment provoked significant cell death ($P<0.001$), which was reversed following addition of recombinant A20. A significant increase in caspase-8 mRNA expression was observed in A20 silenced cells exposed to TRAIL ($P<0.001$, 4 and 24 h).

Conclusion

The data suggest that A20 may offer protection against TRAIL induced apoptosis in pancreatic β-cells.

References

1. Liuwantara *et al*. *Diabetes* 2006.
2. Bernardi *et al*. *Curr Pharm Des* 2012.
3. Bellail *et al*. *Cancer Discov* 2012.

DOI: 10.1530/endoabs.34.OC2.3

OC2.4

Influence of glucocorticoid receptor density on development and remodeling of the heart

Rachel Richardson[1], Eva Rog-Zielinska[2], Adrian Thomson[1], Carmel Moran[1], Gillian Gray[1] & Karen Chapman[1]
[1]The University of Edinburgh, Edinburgh, UK; [2]Imperial College London, London, UK.

Variation in the human glucocorticoid receptor (GR) gene associates with relative glucocorticoid resistance, hypertension and increased cardiovascular disease risk. Mice heterozygous for a null GR mutation ($GR^{+/-}$) are also glucocorticoid resistant with raised circulating glucocorticoid levels and elevated blood pressure in adulthood. We have characterised the cardiac phenotype of these mice throughout development and investigated their response to cardiovascular challenge in adulthood.

$GR^{+/-}$ mice are present at half the expected number at weaning (3 weeks old). Cardiac function is impaired in $GR^{+/-}$ mice at embryonic day (E) 17.5, with Doppler measurements (Visualsonics Vevo770 ultrasound) of blood flow within the left ventricle showing a detrimental increase in the myocardial performance index (representing combined systolic and diastolic function), due to prolonged isovolumetric contraction and relaxation times. This is normalised by postnatal day 7 and remains comparable to WT littermates in adult male $GR^{+/-}$ mice. At E17.5, $GR^{+/-}$ mice lack the normal maturational increase in cardiac mRNA encoding myosin heavy chain α (MHCα) and atrial-natriuretic peptide (ANP). By adulthood, MHCα mRNA levels were equivalent to WT, whilst ANP remained lower ($P<0.05$). Furthermore, cardiomyocyte cross-sectional area was reduced in adult $GR^{+/-}$ mice compared with WT littermates ($P<0.05$) suggesting a potential underlying vulnerability to cardiac challenge. Cardiac remodelling was investigated in adult male mice. Angiotensin II infusion (200 ng/kg per min, 14 days) caused similar increases in heart weight ($P<0.0001$), cardiomyocyte hypertrophy ($P<0.001$) and collagen deposition ($P<0.0001$) in WT and $GR^{+/-}$ mice, indicating that GR haploinsufficiency does not alter pathological cardiac remodelling in this model.

In summary, reduced GR density alters the trajectory of cardiac maturation during development. Whilst compensatory adaptations occur in surviving mice for the functional impairment seen *in utero,* subtle structural and molecular abnormalities remain in adulthood and are likely to contribute to cardiovascular disease risk, in combination with the elevated blood pressure and glucocorticoid levels.

DOI: 10.1530/endoabs.34.OC2.4

OC2.5

β-cells require CFTR for glucose-induced insulin secretion

Jonathan Robinson[1], Rebecca Yates[1], Alan Harper[1] & Catriona Kelly[2]
[1]Keele University, Stoke-on-Trent, UK; [2]University of Ulster, Londonderry, UK.

Background
Cystic fibrosis is an autosomal recessive disease characterised by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The mutations alter fluid secretion in the lungs and digestive systems and the vast majority of patients die from pulmonary disease. CF-related diabetes (CFRD) is the most significant co-morbidity for patients with CF and accelerates lung decline. Recent evidence from animal and cell models has implicated a role for CFTR in the development, organisation and function of the endocrine pancreas and specifically, of the insulin-producing β-cell. This study will address the hypothesis that loss of functional CFTR contributes to the development of CFRD through β-cell dysfunction and apoptosis.
Methods
BRIN–BD11 cells (β-cell line of rat origin) were used for all experiments. Native CFTR was silenced using a siRNA against CFTR (Qiagen). Cell viability was assessed using an MTT assay. Acute glucose-induced insulin secretion was evaluated by exposing cells to rising D-glucose concentrations (1.1, 5.6 and 16.7 mM) for 20 min. Insulin release into the supernatants was measured by ELISA.
Results
Transfection efficiencies of 74 ± 8.2% were achieved ($n=3$). MTT assays found no significant difference in cellular viability between WT and CFTR-deficient cells ($n=4$). WT cells displayed a dose-dependent increase in glucose-induced insulin release. While a significant difference in glucose-induced insulin secretion was not observed at basal glucose concentrations (1.1 mM glucose), CFTR-deficient cells displayed a significant impairment in insulin release in response to intermediate and stimulatory concentrations of glucose (5.6 and 16.7 mM glucose respectively), when compared with WT cells ($n=4$, $P<0.01$–0.001).
Conclusion
CFTR appears to play a significant role in the normal function of pancreatic β-cells. Future work will examine how specific CFTR mutations affect β-cell function and survival.

DOI: 10.1530/endoabs.34.OC2.5

OC2.6

A potential role for food-derived microRNAs in human placental development

Kate Timms, Melissa Westwood & Karen Forbes
University of Manchester, Manchester, UK.

Normal placental and fetal growth are important for neonatal and lifelong health. Placental growth is influenced by endogenous microRNAs (miRs) which regulate translation of their target genes into proteins. Recently, plant miRs from ingested food have been detected in mammalian circulation; maternal fruit and vegetable intake is important for normal development but the underlying mechanisms are not well understood. We hypothesised that miRNAs from maternal dietary fruit and vegetables influence placental growth and therefore investigated whether plant-specific miR-168a has a role in the human placenta.
Using QPCR, miR-168a was found to be present in human maternal serum and placenta. Bioinformatic analysis revealed that multiple components of the epidermal growth factor (EGF) signalling pathway, including the EGF receptor (EGFR), were putative miR-168a targets. EGFR is known to regulate both basal and EGF-stimulated placental growth. The potential role of miR-168a in regulating placental EGFR signalling was assessed using miR-168a mimics (50 nM) to overexpress miR-168a in BeWo choriocarcinoma cells (371-fold increase, $P<0.05$). Western blotting and QPCR revealed that miR-168a overexpression resulted in reduced EGFR protein (62%; $P<0.05$), but not mRNA expression, consistent with known miR actions. Immunofluorescence of the cell cyle marker, Ki67, showed that overexpression of miR-168a decreased both basal (53%; $P<0.05$) and EGF-stimulated (10 nM EGF; 50%; $P<0.05$) BeWo proliferation, consistent with a role for miR-168a in regulating EGFR mediated growth.
In summary, plant-specific miR-168a is present in maternal serum and can be detected in human placenta. The ability of dietary plant-derived miRs to influence gene expression and growth factor actions in human cells and tissue warrants further exploration. Our study suggests that they may have a role in regulating human placental development. Furthermore, the use of miR-168a mimics may have therapeutic potential in cancers and other diseases characterised by dysregulated EGF signalling.

DOI: 10.1530/endoabs.34.OC2.6

Steroids

OC3.1

Lack of 11β-hydroxysteroid dehydrogenase type 1 ameliorates the adverse features of Cushing's syndrome

Stuart Morgan[1], Emma McCabe[1], Laura Gathercole[1], Zaki Hassan-Smith[1], Dean Larner[1], Iwona Bujalska[1], Paul Stewart[1,2], Jeremy Tominson[1] & Gareth Lavery[1]
[1]University of Birmingham, Birmingham, UK; [2]University of Leeds, Leeds, UK.

Glucocorticoids are widely prescribed for their anti-inflammatory properties, but have a significant adverse effect profile, leading to a Cushingoid phenotype. In the present study, we test the hypothesis that reactivation of glucocorticoids, in peripheral tissues by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), is a major determinant of exogenous Cushing's syndrome.
WT, global 11β-HSD1 knockout (GKO), liver-specific 11β-HSD1 knockout (LKO) and fat-specific 11β-HSD1 knockout (FKO) mice were treated with corticosterone (100 μg/ml) or vehicle via drinking water for 5 weeks.
Corticosterone treated WT and global GKO mice had grossly elevated serum corticosterone levels. However, GKO mice were protected from Cushingoid features, as indicated by reversal of glucose intolerance, hyperinsulinaemia, systolic hypertension, increased adiposity, myoatrophy and dermal atrophy. Corticosterone increased expression of the lipolytic enzymes: hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) in adipose tissue of WT mice, paralleled by increased serum free fatty acids (FFAs) and hepatic steatosis, whilst GKO mice were completely protected from the hepatic manifestations of corticosterone treatment. Similarly, in corticosterone-treated FKO mice, reversal of increased ATGL and HSL in adipose tissue, increased serum FFAs and hepatic steatosis was demonstrated, but no protection from the impact of corticosterone was observed in LKO mice.
These data demonstrate that glucocorticoids, reactivated by 11β-HSD1 in peripheral tissues, are a major determinant of Cushingoid features in corticosteroid treated mice. Furthermore, local glucocorticoid regeneration in adipose tissue, and not liver, is central in driving the hepatic manifestations of glucocorticoid excess. 11β-HSD1 is an exciting therapeutic target for patients with Cushing's syndrome.

DOI: 10.1530/endoabs.34.OC3.1

OC3.2

5α-reductase is a regulator of glucocorticoid action and metabolic phenotype in human liver

Maryam Nasiri, Nikolaos Nikolaou, Silvia Parajes, Iwona Bujalska, Laura Gathercole & Jeremy Tomlinson
University of Birmingham, Birmingham, UK.

Patients with GC excess (Cushing's syndrome) develop central obesity, insulin resistance and hepatic steatosis. The A-ring reductases (5α-reductase type 1 (5αR1) and 2 (5αR2)) generate dihydrotestosterone from testosterone, but importantly also inactivate cortisol and are highly expressed in human liver. We propose that 5αR may regulate GC exposure and therefore may modulate metabolic phenotype in human liver.
Primary human hepatocytes and the C3A human hepatoma cell line were incubated with cortisol (0–1000 nM), alone or in combination with the selective 5αR2 inhibitor, Finasteride (500 nM) or non-selective inhibitor, Dutasteride (500 nM) for 24 h. In addition, C3A cells (which express 5αR1, but not 5αR2), were transfected with a plasmid containing either WT, or inactivated mutant (R246Q) 5αR2. The functional impact of these manipulations was assessed through real-time PCR based gene expression, enzyme activity assays using both gas and liquid chromatography/mass spectrometry and functional assessments of *de novo* lipogenesis (DNL).
Cortisol decreased DNL in a dose-dependent manner (e.g. 85.66.6% (100 nM), 73.57.9% (250 nM), 55.045.6% (1000 nM), $P<0.05$). 5αR2 over-expression increased DHT generation and cortisol clearance and in the absence of cortisol, did not alter rates of DNL. However, in the presence of cortisol, 5αR2 restored DNL to levels observed in untreated controls (e.g. 61.9 ± 7.6% (cortisol) vs 103.8 ± 8.8% (5αR2 + cortisol), $P<0.05$, control = 100%). Complementary experiments using the R246Q 5αR2 construct did not alter cortisol-mediated suppression of DNL. Furthermore, both Finasteride and Dutasteride augmented the action of cortisol to supress DNL in primary cultures of human hepatocytes (e.g. 88.3 ± 5.3 vs 76.9 ± 5.2%, cortisol vs cortisol + finasteride, $P=0.05$).
We have demonstrated that manipulation of 5αR activity can regulate metabolic phenotype in human liver. Further clinical studies are now warranted, but this may have significant clinical implications for those patients with mutations in 5αR2 and those prescribed 5αR inhibitors.

DOI: 10.1530/endoabs.34.OC3.2

OC3.3

The 21-hydroxylase pseudogene may have a role in induction of tolerance to steroidogenic machinery

Anna Louise Mitchell[1], Ingeborg Bronstad[2], Anette Boe Wolff[2], Alekhya Narravula[1], Beate Skinningsrud[3], Eystein S Husebye[2] & Simon H S Pearce[1]
[1]Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK; [2]Department of Medical Genetics, Haukeland University Hospital, Bergen, Norway; [3]Department of Medical Genetics, Oslo University Hospital, Oslo, Norway.

The 21-hydroxylase (21OH) gene, *CYP21A2*, encodes the 21OH steroidogenic enzyme which is the primary autoantigen in autoimmune Addison's disease (AAD). It is located on chromosome *6p21*, in a copy number repeat termed RCCX, adjacent to the 21OH pseudogene (*CYP21A1P*). *CYP21A1P* is highly homologous to *CYP21A2* but contains an 8 bp deletion in exon 3 (*707-714delGAGACTAC*) which results in a frameshift. The predicted protein product is therefore truncated and not enzymatically functional. We hypothesise that *CYP21A1P* transcripts are expressed in human thymus to induce immune tolerance to the steroidogenic machinery, without allowing generation of glucocorticoid.

We used a PCR method to determine whether individuals with AAD from the UK and Norway are more likely to have no copies of CYP21A1P compared to controls. HLA data available for the Norwegian cohort were then analysed to determine whether CYP21A1P absence is associated with the 8.1 ancestral HLA haplotype (8.1 AH). Quantitative PCR (qPCR) was then used to determine whether CYP21A1P transcripts are detectable in thymus tissue from seven individuals (age 8 days to 16 months).

Of 315 UK AAD individuals, 55 (17.5%) had no *CYP21A1P* genomic DNA copies compared with 19/627 (3.0%) controls (P 8.1×10^{-15}, OR 6.77 (95% CI 3.94–11.63)). In the Norwegian cohort, a less marked association was observed: 42/319 (13.2%) AAD compared to 19/413 (4.6%) controls (P 3.2×10^{-5}, OR 3.14 (1.79–5.52)). HLA haplotype reconstruction showed that *CYP21A1P* absence is strongly associated with the 8.1 AH in both cases (P 9.6×10^{-7}) and controls (P 1.2×10^{-7}). qPCR demonstrated the presence of *CYP21A1P* transcripts at low levels in thymus tissue (mean 8 copies/μl, range 3–24).

This study has demonstrated that absence of genomic *CYP21A1P* is associated with AAD and is likely tagging the HLA 8.1 AH. *CYP21A1P* transcripts are present in thymus tissue and therefore could have a role in induction of tolerance. This exciting finding suggests a functional role for *CYP21A1P* and is consistent with the conservation of the pseudogene in other primates and rodents.

DOI: 10.1530/endoabs.34.OC3.3

OC3.4

Pre-receptor glucocorticoid metabolism across human ageing: the impact of gender and menopausal status

Zaki Hassan-Smith, Stuart Morgan, Mark Sherlock, Beverly Hughes, Gareth Lavery, Jeremy Tomlinson & Paul Stewart
University of Birmingham, Birmingham, UK.

Introduction

There is growing evidence that 11β-HSD1 expression/activity increases with age in key target tissues including adipose tissue, bone, and skin, implicating local amplification of glucocorticoids in the pathophysiology of related disease. We have previously shown that 11β-HSD1KO mice are protected from both the adverse metabolic effects of excess glucocorticoids and age-associated muscle weakness. We investigated changes in global activity and skeletal muscle gene expression of 11β-HSD1 with human ageing.

Methods

135 healthy volunteers (women $n=77$, men $n=58$, aged 20–80 years) were recruited. DEXA body composition analysis, strength testing, serum and 24-h urine collection (analysed by GC/MS) and vastus lateralis muscle biopsies (92 genes analysed by microfluidic array) were performed.

Results

Skeletal muscle 11β-HSD1 expression increased with age in women. Furthermore, women aged >70 years had increased urine (THF+5αTHF)/THE ratios vs women in their 1920s. 11β-HSD1 expression was positively correlated with serum gonadotrophins, body fat and total cholesterol and was negatively correlated with bone mineral content, grip strength and serum IGF1, in women. Total F metabolites were positively correlated with fat mass, in both sexes. In skeletal muscle, we observed age-related changes in genes encoding proteins with functions in pre-receptor GC metabolism (11β-HSD1, H6PDH) cell stress response (GADD45a, HIF-1α, CDKN1A, HSP90B1, SIRT3) and proteolysis (PSMA2, PSMD4).

Conclusion

Skeletal muscle 11β-HSD1 expression increases with age in women, and this change may be driven by the menopause. The therapeutic potential of selective inhibitors of 11β-HSD1 in ameliorating the adverse metabolic and body composition profile associated with ageing and the menopause remains to be determined.

DOI: 10.1530/endoabs.34.OC3.4

OC3.5

Linking GWAS to gene function: *CYP17A1* in hypertension

Louise Diver[1], Scott MacKenzie[1], Frances McManus[1], Marie Freel[1], Elaine Friel[1], Robert Fraser[1], Neil Hanley[3], John Connell[2] & Eleanor Davies[1]
[1]University of Glasgow, Glasgow, UK; [2]University of Dundee, Dundee, UK; [3]University of Manchester, Manchester, UK.

Recent genome-wide association studies (GWAS) implicate the *CYP17A1* locus in human blood pressure regulation. This gene is important in steroidogenesis, regulating both glucocorticoid and androgen synthesis through catalysis of 17α-hydroxylation and 17,20 lyase reactions. We hypothesised that functional *CYP17A1* polymorphisms linked to those identified by GWAS influence blood pressure levels. We therefore screened the entire *CYP17A1* locus by direct sequencing, identifying possible functional variants through *in vitro* analysis and correlating genotype with intermediate corticosteroid phenotype in a hypertensive cohort.

Examination of polymorphic variation to establish patterns of linkage disequilibrium across the *CYP17A1* locus was conducted in a normotensive cohort. Variants located in the 5′ regulatory region were prioritised for further investigation. Bioinformatic analysis coupled with *in vitro* reporter gene assays confirmed that single base changes at three polymorphic sites each altered transcriptional activity. Association between genotype and intermediate corticosteroid phenotype was explored using 24-h urinary metabolite excretion data from a cohort of 232 hypertensive subjects. When stratified by genotype and gender, increased cortisol excretion rates were found to associate with the minor allele at rs248658 in males ($P=0.05$) and at rs2150927 in females ($P=0.04$). Furthermore, ratios of selected corticosteroid intermediary metabolites (e.g. THDOC:THS) were significantly reduced in the presence of the rs2150927 minor allele, in a manner indicative of increased 17α-hydroxylase efficiency ($P=0.02$). Aldosterone excretion was also significantly elevated in individuals with CC genotype at rs138009835 ($P=0.05$); we propose an indirect genotype-dependent effect.

This study identifies that polymorphisms linked to those implicated in previous GWAS significantly alter *CYP17A1* transcription. Furthermore, these variants associate with corticosteroid intermediate phenotypes in a hypertensive population, implying functional effect and a mechanism for the development of hypertension. Further studies will determine whether observed changes in transcriptional activity are the direct result of altered transcription factor binding at polymorphic sites.

DOI: 10.1530/endoabs.34.OC3.5

OC3.6

Targeting of lysyl oxidase by steroids to reduce peritoneal fibrosis and scarring

Christopher Harlow[1], Xuan Wu[1], Marielle van Deemter[1], Fiona Gardiner[1], Craig Poloand[2], Rebecca Green[1], Sana Sarvi[1], Karl Kadler[3], Yinhui Lu[3], J Ian Mason[1], Hilary Critchley[1] & Stephen Hillier[1]
[1]MRC/University of Edinburgh Centre for Reproductive Health, Edinburgh, UK; [2]MRC/University of Edinburgh Centre for Inflammation Research, Edinburgh, UK; [3]University of Manchester, Wellcome Trust Centre for Cell-Matrix Research, Manchester, UK.

Abdominal surgery and diseases such as endometriosis cause persistent abdominal adhesions leading to pelvic pain, infertility and in severe cases, bowel obstruction. Current treatments are ineffective and the aetiology is unclear, although excessive collagen deposition is a consistent feature. Lysyl oxidase (LOX) is a key enzyme required for crosslinking and deposition of insoluble collagen.

10–12 weeks old female C57Bl/6 mice (3–10 mice per group) were treated with 25 ng multiwalled carbon nanotubes (NT) as an i.p. insult to induce fibrosis, together with chemical (β-aminoproprionitrile – BAPN) or miRNA LOX inhibitors, progesterone (Prog) or dexamethasone (Dex) for up to 7 days. Fibrotic lesion area and Picrosirius red staining of the diaphragm, and mRNA expression of fibrosis-related genes in abdominal wall peritoneal mesothelial cells (PMC) were

measured. Effects of BAPN and DEX on collagen fibre alignment were observed by transmission electron microscopy (TEM). Isolated PMC were cultured with and without interleukin 1α (IL1α) and Prog to determine effects on *lox* mRNA *in vitro*. NT induced extensive fibrosis and collagen deposition on the diaphragm, that was significantly ameliorated by BAPN, LOX miRNA, Prog or Dex. BAPN and Dex disrupted ordered collagen fibre bundles induced by NT as observed by TEM. Expression of *lox*, *col1a1*, *col3a1* and *bmp1* mRNA in abdominal wall PMC were all significantly increased by NT, but this effect was inhibited by treatment for up to 7 days with Prog or Dex. Physiological levels of Prog significantly inhibited IL1α induced *lox* expression by PMC *in vitro*.

Our results suggest targeting of LOX in the peritoneum may ameliorate fibrosis and the development of adhesions, and point to simple, naturally occurring or widely used steroids as potential agents to prevent adhesion formation following abdominal surgery.

DOI: 10.1530/endoabs.34.OC3.6

Thyroid and bone

OC4.1

Adaptor protein-2 sigma subunit mutations causing familial hypocalciuric hypercalcaemia type 3 exert dominant-negative effects

Sarah Howles, M Andrew Nesbit, Fadil Hannan, Angela Rogers, Sian Piret, Nigel Rust & Rajesh Thakker
University of Oxford, Oxford, UK.

Adaptor protein-2 (AP2) is a heterotetramer of α, β, μ, and σ subunits that is pivotal in clathrin-mediated endocytosis and facilitates internalisation of plasma membrane constituents such as the calcium-sensing receptor (CaSR). AP2 σ subunit (AP2σ) missense mutations (Arg15Cys, Arg15His and Arg15Leu) result in familial hypocalciuric hypercalcaemia type 3 (FHH3) and decrease the sensitivity of CaSR-expressing cells to changes in extracellular calcium concentration. FHH3 is an autosomal dominant disorder and we hypothesized that AP2σ mutations may exert dominant-negative loss-of-function effects on the AP2 complex. We therefore studied the effect of increasing levels of WT and mutant AP2σ in CaSR-expressing cells by transient transfection with WT or mutant AP2S1-pBI-CMV2-GFP bidirectional expression construct that expresses GFP and AP2σ in a 1:1 ratio. Populations of cells with GFP expression varying from 1- to 32-fold over baseline, and thus AP2σ expression, were assessed by measurement of intracellular calcium responses to changes in extracellular calcium concentration, enabling calculation of the half-maximal effective concentration (EC_{50}) values for each cellular population. Linear regression of mean EC_{50} values ($n=8$) for increasing expression levels of WT AP2σ demonstrated no deviation from zero ($P=0.28$, $R^2=0.27$), indicating that progressive over-expression of WT AP2σ does not affect the function of the AP2 complex in CaSR signaling. In contrast, linear regression of mean EC_{50} values ($n=8$) for increasing expression levels of mutant AP2σ demonstrated a positive incline (Cys15 $P=0.014$, $R^2=0.81$; His15 $P=0.0083$, $R^2=0.86$; Leu15 $P=0.028$, $R^2=0.74$) indicating that increasing levels of mutant AP2σ proteins progressively impair intracellular responses to variations in extracellular calcium in CaSR-expressing cells. Thus, our results, demonstrate a likely dominant-negative effect of the AP2σ mutants (σ^m), whereby the mutant heterotetramer ($AP2\alpha\beta\mu\sigma^m$) interferes with the binding of WT heterotetramer ($AP2\alpha\beta\mu\sigma$) to the activated CaSR, and provide a mechanistic explanation for the dominant inheritance pattern of FHH3.

DOI: 10.1530/endoabs.34.OC4.1

OC4.2

PHOSPHO1: roles beyond skeletal mineralisation

Karla Oldknow[1], Nik Morton[2], Manisha Yadav[3], Sophie Rajoanah[2], Carmen Huesa[1], Lutz Bunger[5], Derek Ball[4], Mathieu Ferron[6], Gerard Karsenty[7], Vicky MacRae[1], Jose Luis Millan[3] & Colin Farquharson[1]
[1]The Roslin Institute, Edinburgh, UK; [2]The University of Edinburgh, Edinburgh, UK; [3]Sanford Children's Health Research Center, La Jolla, California, USA; [4]Heriot-Watt University, Edinburgh, UK; [5]SRUC, Edinburgh, UK; [6]Institut de Recherches Cliniques de Montréal Montréal, Québec, Canada; [7]Columbia University, New York, New York, USA.

Advances in genetic approaches to bone physiology have expanded our understanding of the mechanisms by which bone and energy homeostasis interact. PHOSPHO1, a bone specific phosphatase is essential for the initiation of bone mineralisation. Here we now show that *Phospho1* ablation confers a remarkable protection against obesity and diabetes in mice. To understand the mechanism whereby *Phospho1* impacts metabolism, microarray analysis of osteoblasts, the primary site of *Phospho1* expression was performed. *Esp* (encoding the phosphatase OST-PTP) which controls hormonally active osteocalcin secretion, was 20-fold more highly expressed in $Phospho1^{-/-}$ osteoblasts ($P<0.05$). Conversely, *Esp* mRNA was decreased in *Phospho1* overexpressing osteoblasts ($P<0.001$). Unexpectedly, serum levels of uncarboxylated and undercarboxylated osteocalcin were normal suggesting an osteocalcin-independent mechanism of PHOSPHO1 regulated energy metabolism. 120 day-old $Phospho1^{-/-}$ mice were hypoglycaemic (WT 9.48±0.31 mmol/l, $Phospho1^{-/-}$ 8.30±0.26 mmol/l; $P<0.01$) and showed improved glucose and insulin tolerance compared to WT mice ($P<0.05$). These observations were consistent with the finding of smaller (mg/g BW) subcutaneous (WT 4.51±0.37, $Phospho1^{-/-}$ 2.79±0.42; $P<0.01$) and mesenteric (WT 13.2±1.34, $Phospho1^{-/-}$ 5.56±1.61; $P<0.01$) fat deposits noted in $Phospho1^{-/-}$ mice at necropsy and confirmed by MRI. Remarkably, $Phospho1^{-/-}$ mice resisted the pronounced weight gain (WT 38.0±1.54 g, $Phospho1^{-/-}$ 32.4±1.26 g; $P<0.05$) and diabetes (WT 10.3±0.53 mmol/l, $Phospho1^{-/-}$ 9.27±0.77 mmol/l; $P<0.05$) exhibited by WT mice when fed a chronic high fat diet (HFD; 14 weeks, 58% kcal as fat) and this was not explained by altered activity. Histology revealed smaller epididymal adipocytes, decreased fat content, decreased pancreatic islet number and increased mitochondria number in brown fat ($P<0.05$). However, no differences were observed in brown fat specific genes including *Ucp1* suggesting canonical thermogenesis does not underlie metabolic protection. Our findings indicate *Phospho1* deficiency improves the metabolic profile of mice *in vivo* and confers resistance to obesity and diabetes most likely through a primary effect on bone metabolism/turnover.

DOI: 10.1530/endoabs.34.OC4.2

OC4.3

TRα mutation causes a severe and thyroxine-resistant skeletal dysplasia

J H Duncan Bassett[1], Alan Boyde[2], Tomas Zikmund[3], Holly Evans[4], Peter I Croucher[5], Xuguang Zhu[6], Jeong Won Park[6], Sheue-yann Cheng[6] & Graham R Williams[1]
[1]Imperial College London, London, UK; [2]Queen Mary University of London, London, UK; [3]Brno University of Technology, Brno, Czech Republic; [4]University of Sheffield, Sheffield, UK; [5]Garvan Institute of Medical Research, Sydney, New South Wales, Australia; [6]National Cancer Institute, Bethesda, Maryland, USA.

A new genetic disorder has recently been identified that results from mutation of THRA, encoding thyroid hormone receptor α1 (TRα1). Affected children have a high serum T_3:T_4 ratio, constipation and a variable intellectual deficit, but exhibit a consistently severe skeletal dysplasia. Similar to these patients, $Thra1^{PV/+}$ mice harbour a mutation that disrupts the C-terminal α-helix of TRα1 and express a dominant negative receptor. Thus, $Thra1^{PV/+}$ mice represent an excellent disease model for this new disorder. We hypothesized $Thra1^{PV/+}$ mice could be used to predict the skeletal consequences of human *THRA* mutations and investigate the effects of T4-treatment. We determined the adult skeletal phenotype in $Thra1^{PV/+}$ mice and investigated the response to treatment with a supra-physiological dose of thyroxine (1.2 μg/ml in drinking water from 4 until 20 weeks of age).

Adult $Thra1^{PV/+}$ mice had persistently delayed ossification and short stature but normal bone strength despite high bone mass, suggesting that patients with *THRA* mutations are unlikely to have an increased risk of fracture. By contrast, gross morphological abnormalities of the bones and joints predict a likely predisposition to osteoarthritis. Although T4-treatment completely suppressed TSH secretion, it had no effect on skeletal maturation, linear growth or bone mineralization, thus demonstrating profound tissue resistance to thyroid hormone in the skeleton of $Thra1^{PV/+}$ mice. Nevertheless, prolonged T4-treatment resulted in abnormally increased bone stiffness and strength due to progressive enlargement of cortical bone, suggesting the potential for detrimental long-term consequences. Furthermore, despite identical T4-treatment, $Thra1^{PV/+}$ mice exhibited a blunted rise in circulating thyroid hormones, suggesting increased thyroid hormone metabolism in $Thra1^{PV/+}$ mice.

Our studies establish that TRα1 has an essential role in the developing and adult skeleton, and predict that, in patients with *THRA* mutations, the severity of skeletal dysplasia and responsiveness to T4-treatment will correlate with the activity of the mutant TRα1.

DOI: 10.1530/endoabs.34.OC4.3

OC4.4

Thyroid hormones stimulate osteoclastogenesis via TRα-dependent actions in osteoblasts

John G Logan, J H Duncan Bassett & Graham R Williams
Imperial College London, London, UK.

Thyrotoxicosis results in osteoporosis and thyroid hormone (T_3) stimulates osteoclastic bone resorption by unknown mechanisms. We previously demonstrated that knockout mice lacking thyroid hormone receptor α (TRα$^{0/0}$) are euthyroid but have high bone mass, whereas mice lacking TRβ (TRβ$^{-/-}$) are thyrotoxic and have osteoporosis. Tartrate resistant acid phosphatase (TRAcP) staining revealed osteoclast numbers were reduced by 13% ($P<0.05$) in TRα$^{0/0}$ mice, but increased by 20% ($P<0.05$) in TRβ$^{-/-}$ mice, suggesting T_3 acts via TRα to stimulate osteoclastogenesis and bone resorption.
To test this hypothesis, we stimulated bone marrow (BM) from WT, TRα$^{0/0}$ and TRβ$^{-/-}$ mice with M-CSF (25 ng/ml) and RANKL (10 ng/ml) in the absence or presence of T_3 (100 nM). T_3 had no effect on the number, size or survival of osteoclasts of any genotype demonstrating T_3 does not exert direct actions in osteoclasts. Despite this, threefold ($P<0.001$) greater numbers of osteoclasts formed in cultures from TRα$^{0/0}$ mice compared to WT or TRβ$^{-/-}$, indicating impaired osteoclast precursor cell differentiation *in vivo* in TRα$^{0/0}$ mice. WT, TRα$^{0/0}$ or TRβ$^{-/-}$ BM was co-cultured with WT osteoblasts in the absence and presence of T_3, but without addition of M-CSF and RANKL. There was a 35–66% ($P<0.05$) increase in osteoclast number and TRAcP activity following T_3 treatment in all these co-cultures, indicating impaired osteoclastogenesis in TRα$^{0/0}$ mice does not result from an osteoclast defect. We next co-cultured WT BM with WT, TRα$^{0/0}$ or TRβ$^{-/-}$ osteoblasts in the absence or presence of T_3. T_3 treatment resulted in a 45–68% increase ($P<0.01$) in osteoclast number and TRAcP activity when WT BM was co-cultured with WT and TRβ$^{-/-}$ osteoblasts. By contrast, few osteoclasts formed in co-cultures of WT BM with TRα$^{0/0}$ osteoblasts in the absence or presence of T_3.
Overall, these data demonstrate that T_3 stimulates bone resorption indirectly via TRα-dependent actions in osteoblasts.

DOI: 10.1530/endoabs.34.OC4.4

OC4.5

A mutation in the 5′-UTR of *GNA11* causes familial hypocalciuric hypercalcemia type 2 due to reduced translational efficiency

Sarah Howles[1], MAndrew Nesbit[1], Fadil Hannan[1], Sian Piret[1], Treena Cranston[2], Mushtaqur Rahman[3] & Rajesh Thakker[1]
[1]University of Oxford, Oxford, UK; [2]Oxford University Hospitals NHS Trust, Oxford, UK; [3]North West London Hospitals NHS Trust, London, UK.

The calcium-sensing receptor (CaSR) is a guanine-nucleotide-binding protein (G-protein)-coupled receptor that has a central role in calcium homeostasis. Loss-of-function mutations of the CaSR result in familial hypocalciuric hypercalcemia type 1 (FHH1) and loss-of-function coding mutations in the CaSR-associated G-protein subunit $G\alpha_{11}$ have been reported to cause FHH2 in only two patients to date. The aim of our study was therefore to characterise additional *GNA11* mutations associated with FHH2. We undertook DNA sequence analysis of the 1077-bp coding region, 12 exon–intron boundaries and 5′-UTR of *GNA11* in 40 unrelated hypercalcaemic patients who did not have *CaSR* mutations. This identified in one patient, a 40-bp deletion in the 5′-UTR, encompassing positions −43 to −4 with respect to the ATG, of *GNA11*. To investigate the effect of this mutation on the translational efficiency of *GNA11,* luciferase (*luc*) reporter expression vectors were constructed containing *luc* under the control of either the WT *GNA11* promoter and 5′-UTR or the WT *GNA11* promoter and mutant 5′-UTR. Transient transfection of HEK293 cells stably expressing the CaSR with these constructs demonstrated that the mutant 5′-UTR sequence resulted in a >80% reduction in luciferase activity compared to the WT 5′ UTR sequence (WT, luciferase activity fold change compared to untransfected cells = 70.81 ± 3.77; 5′ UTR deletion, luciferase activity fold change compared to untransfected cells = 12.89 ± 0.72, $P<0.0001$). Thus, our results demonstrate that the FHH2-associated 40-bp deletion in the 5′-UTR of *GNA11* significantly decreases the translational efficiency of $G\alpha_{11}$ and that FHH2 may be due to haploinsufficiency rather than a dominant-negative effect. These studies, which have identified the first non-coding FHH2-causing mutation in *GNA11,* provide new insights into the role of $G\alpha_{11}$ in CaSR signalling and the mechanisms causing FHH2.

DOI: 10.1530/endoabs.34.OC4.5

OC4.6

An ENU-induced Tyr265Stop mutation in Polg2 is associated with renal calcification in RCALC2 mice

Caroline Gorvin[1], Sian Piret[1], Bushra Ahmad[1], Michael Stechman[1], Nellie Loh[1], Tertius Hough[2], Paul Leo[3], Mhairi Marshall[3], Siddharth Sethi[4], Liz Bentley[4], Anita Reed[1], Paul Christie[1], Michelle Simon[4], Ann-Marie Mallon[4], Matthew Brown[3], Roger Cox[4], Steve Brown[4] & Rajesh Thakker[1]
[1]Academic Endocrine Unit, Oxford Centre for Diabetes, Endocrinology and Metabolism, Oxford, UK; [2]Mary Lyon Centre, Medical Research Council, Harwell, UK; [3]The University of Queensland Diamantina Institute, Translational Research Institute, Wooloongabba, Queensland, Australia; [4]Mammalian Genetics Unit, Medical Research Council, Harwell, UK.

Renal calcification (nephrocalcinosis), which has a multi-factorial etiology involving environmental and genetic determinants, affects ∼8% of adults by 70 years. Nephrocalcinosis may occur as a familial disorder in ∼65% of patients, and in 70% of patients, nephrocalcinosis may be associated with endocrine and metabolic disorders that include primary hyperparathyroidism, renal tubular acidosis, hypercalciuria, cystinuria, and hyperoxaluria. Investigations of families with hereditary nephrocalcinosis have identified the involvement of genes encoding the chloride/proton antiporter CLC-5, and claudin-16, but further progress has been limited as large families are not available for genetic linkage and mapping studies. To overcome this limitation, we embarked on establishing mouse models for nephrocalcinosis by performing abdominal X-rays to identify renal opacities in 1745 12-month-old male mice derived by *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis. This identified a mouse with renal calcification, designated RCALC2, that was inherited as an autosomal dominant trait. Genome-wide mapping located the *Rcalc2* locus to a ∼16 Mbp region on chromosome 11D-E2 and whole-exome sequence analysis revealed a heterozygous c.C795A substitution in polymerase gamma-2, accessory subunit, *Polg2*, that resulted in a nonsense mutation (Tyr265Stop). Further analysis of *Polg2*, which encodes the minor subunit of the mitochondrial DNA polymerase and is required for strengthening polymerase–mitochondrial DNA interactions, revealed RCALC2 mouse kidneys to have lower POLG2 mRNA and protein expression, and co-immunoprecipitation studies showed the mutant to have a loss of interaction with the major polymerase subunit, POLG. This was not associated with a change in mitochondrial DNA content in RCALC2 kidneys, or mitochondrial function such as reactive oxygen species (ROS) generation or apoptosis, which was assessed by Oxiselect Intracellular ROS and Caspase-Glo-3/7 luminescent assays, respectively. In conclusion, RCALC2 is a novel mouse model of renal calcification that may reveal new functions for POLG2 in endocrine and metabolic diseases associated with nephrocalcinosis.

DOI: 10.1530/endoabs.34.OC4.6

Pituitary

OC5.1

Increased frequency and earlier onset of pituitary tumours in mice deleted for a multiple endocrine neoplasia type 1 allele and null for prolyl hydroxylase domain protein 1 (*Men1*$^{+/-}$/*Phd1*$^{-/-}$)

Mark Stevenson, Sian Piret, Mahsa Javid, Tammie Bishop, Anita Reed, Gerard Walls, Katie Gaynor, Paul Newey, Paul Christie, Lynn Nicholls, Peter Ratcliffe & Rajesh Thakker
University of Oxford, Oxford, UK.

Cumulative genetic abnormalities within an oncogenic pathway may contribute to earlier onset or increased aggressiveness of cancers. An example in human and murine cancer is the dysregulation of Wnt signalling by inactivation of the adenomatous polyposis coli (APC) gene that results in accumulation of nuclear β-catenin and earlier onset of renal cell carcinoma, which can be accelerated by p53-deficiency. We therefore investigated the effects of such cumulative genetic abnormalities in the development of neuroendocrine tumours (NETs), which occur as part of multiple endocrine neoplasia type 1 (MEN1), associated with pancreatic NETs, pituitary NETs and parathyroid tumours; and von Hippel Lindau (VHL) syndrome associated with pancreatic NETs, phaeochromocytomas, renal cancers and haemangioblastomas of the retina and CNS. MEN1 includes mutation of the *Men1* gene that encodes menin, a tumour suppressor regulating transcription and genome stability; and VHL tumour development involves a tumour suppressor pathway, including hypoxia-inducible factor-1α (HIF-1α) that is post-translationally modified by prolyl hydroxylase domain proteins (PHDs) to promote HIF-1α ubiquitination and proteosomal degradation. To investigate for cumulative effects we interbred *Men1*$^{+/-}$/*PHD1*$^{+/-}$ mice and studied them for development of NETs. A total of 108 mice comprising 62 *Men1*$^{+/-}$/*PHD1*$^{+/+}$ and 46 *Men1*$^{+/-}$/*Phd1*$^{-/-}$ mice were generated and by

9–12 months of age pituitary tumours, which consisted of prolactinomas and somatotrophinomas with loss of menin expression, had developed in 12 of 46 (26.1%) *Men1*$^{+/-}$/*Phd1*$^{-/-}$ mice compared with four of 62 (6.5%) *Men1*$^{+/-}$ *PHD1*$^{+/+}$ mice ($P<0.01$). Furthermore pituitary tumour development occurred earlier in *Men1*$^{+/-}$/*Phd1*$^{-/-}$ mice such that four of 24 (16.7%) mice had tumours at 9 months of age compared with zero of 38 (0%) *Men1*$^{+/-}$/*PHD1*$^{+/+}$ mice ($P<0.02$). Pancreatic NET development in both groups was similar. Thus, our studies show that loss of *Men1* and *PHD1* may act in an additive manner to result in earlier onset and increased frequency of pituitary tumours.

DOI: 10.1530/endoabs.34.OC5.1

OC5.2

Implications of the upregulation of lysine specific demethylase 1 in the pathogenesis of pituitary adenomas

Iain Thompson, Min He, Erin Clark, Shuyun Xu, Dan Tang, Edward Laws, Yujiang Shi, Rona Carroll & Ursula Kaiser
Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA.

The pathogenesis of pituitary adenomas is poorly understood. One of the first genetic abnormalities identified in association with pituitary adenomas occurs in patients with multiple endocrine neoplasia type-1 (MEN-1), due to mutations in the *MEN1* gene, encoding menin. A tumor suppressor, menin associates with histone methyltransferase complexes to change the expression of cyclin-dependent kinase (CDK) inhibitor genes, which may serve as an underlying epigenetic mechanism important in the control of pituitary cell proliferation. LSD1 and LSD2 are flavin-dependent monoamine oxidases, functioning to demethylate histone H3K4. We hypothesized that the histone methylation status of specific CDK inhibitor genes, modulated by LSD1 and LSD2, might serve as an underlying epigenetic mechanism important in pituitary tumor pathogenesis. We studied the expression of LSD1 and LSD2 in ten pituitary adenomas (from Partners Tissue Bank) and seven normal pituitaries (from autopsy specimens). LSD1 and LSD2 mRNA expression, as well as LSD1 protein levels, were significantly higher in the adenomas compared to controls. The LSD inhibitor, tranylcypromine, significantly reduced the proliferation rates of GH3 somato-lactotrope- and LβT2 gonadotrope-derived cell lines. Specific, lentiviral-delivered shRNA against LSD1, but not LSD2, reduced LβT2 cell proliferation, compared to scramble shRNA controls, suggesting that the proliferation phenotype is LSD1 specific. After LSD1 knock-down (KD) in LβT2 cells, levels of the *CIP/KIP* family of CDK inhibitors, p27 and p57 (but not p21) were significantly increased. No changes in mRNA levels of members of the *INK4a/ARF* family of CDK inhibitors (p15, p16, and p18) were observed. ChIP analysis of LSD1 KD LβT2 cell chromatin, immunoprecipitated with an anti-H3K4Me2 antibody, revealed an increase in H3K4Me2 associated with the p27 gene promoter compared to control cells. These findings suggest a role for LSD1 in pituitary tumorigenesis through effects on cellular proliferation, likely mediated through changes in histone methylation status at the promoters of CDK inhibitors.

DOI: 10.1530/endoabs.34.OC5.2

OC5.3

The Wnt/β-catenin effector Tcf3/TCF7L1 is required for normal hypothalamic–pituitary development and mutation in this gene are associated to congenital hypopituitarism

Carles Gaston-Massuet[1], Mark McCabe[2], Mehul Dattani[2] & J P Martinez-Barbera[2]
[1]William Harvey Research Institue, Centre for Endocrinology, London, UK; [2]ICH, UCL, London, UK.

Aberrant development of the pituitary gland can result in the clinical manifestation of hypopituitarism. The Wnt/β-catenin pathway has been shown to be involved in normal organogenesis, terminal differentiation and the aetiology of pituitary tumours. However, the specific developmental roles during hypothalamic–pituitary development of some of the Wnt/β-catenin effectors, such as *Tcf3*, have been hampered due to the early lethality of null embryos for this gene. To overcome this, we have conditionally deleted *Tcf3* (*Tcf3*$^{Fl/-}$;*Hesx1*$^{Cre/+}$) from the *Hesx1*-expressing cells within the early forebrain and developing pituitary gland. A low proportion of *Tcf3*$^{Fl/-}$;*Hesx1*$^{Cre/+}$ animals exhibit dwarfism indicating that deficiency in *Tcf3* can lead to hypopituitarism in mice. Analyses of *Tcf3*$^{Fl/-}$;*Hesx1*$^{Cre/+}$ mutant embryos reveals a mild hyperplasia of the pituitary gland, sometimes with the mis-location of the pituitary in the pharyngeal cavity. We show that *Tcf3* has a dual function and it is required in both the ventral diencephalon (VD) and anterior pituitary gland (AP). In the VD, absence of *Tcf3* results in aberrant VD signaling with rostrally expanded *Fgf10* and *BMP4* expression domains, leading to a broader region of the oral ectoderm being specified into Rathke's pouch. Within the developing AP, absence of *Tcf3* results in increased mitotic index of periluminal Rathke's pouch progenitors, further exacerbating the AP-hyperplasia. To assess if TCF3 is required to mediate transcriptional activation or repression of Wnt/β-catenin pathway, we studied a second murine mutant (*Tcf3*$^{\Delta N/\Delta N}$) expressing a mutant TCF3 lacking the β-catenin interacting domain, and therefore acting as a constitutive repressor. Interestingly, *Tcf3*$^{\Delta N/\Delta N}$ embryos exhibit normal development of both the prospective hypothalamus and the pituitary gland throughout all developmental stages, indicating that TCF3-repressing activity is essential for hypothalamic–pituitary development. Providing a translational impact to this research, we report the identification of two novel mutation in *hTCF3* that compromises TCF3-repressing activity in a patient with septo-optic dysplasia (SOD), suggesting a causative role of *TCF3* in SOD. In summary, our research demonstrates a critical role for the Wnt/β-catenin effector *Tcf3* during early development of the pituitary–hypothalamic axis in mice and humans.

DOI: 10.1530/endoabs.34.OC5.3

OC5.4

GnRH pulse frequency-dependent regulation of ICER, a modulator of FSHβ transcription, is attenuated by MEKI/II blockade

Iain Thompson, Nick Ciccone, Shuyun Xu, Qiongjie Zhou, Sofiya Zaytseva, Rona Carroll & Ursula Kaiser
Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA.

The pulsatile release of GnRH regulates the synthesis and secretion of pituitary FSH and LH. Two transcription factors, the cAMP response element binding protein (CREB) and inducible cAMP early repressor (ICER), have been implicated in the regulation of rat FSHβ gene expression. We hypothesized that CREB and ICER are activated by distinct signaling pathways in response to pulsatile GnRH to modulate FSHβ gene expression, which is preferentially stimulated at low (every 120 min) vs high (every 30 min) pulse frequencies. Using the LβT2 gonadotrope-derived cell line, we have shown previously that at low GnRH pulse frequencies, PKA is activated, leading to CREB phosphorylation and subsequent FSHβ transcription. In contrast, ICER expression is preferentially stimulated by GnRH at high pulse frequencies. In static culture, GnRH stimulation of LβT2 cells resulted in a time-dependent increase in ICER mRNA at 12 and 24 h, as measured by qRT-PCR. Pharmacological blockade of MEKI/II with two selective inhibitors, U0126 and PD0325901, significantly attenuated GnRH-stimulated ICER induction in a dose-dependent fashion, whereas PKC (GF109203X), CamKII (KN-93) and PKA (H89) inhibitors had minimal effects. Similarly, MEKI/II inhibition attenuated GnRH induction of ICER protein as determined by western blot analysis. Importantly, MEKI/II inhibition with PD0325901 in perifused LβT2 cells stimulated with pulsatile GnRH abrogated ICER induction at high GnRH pulse frequency, whereas stimulation of ICER at low GnRH pulse frequency was unaffected. MEKI/II inhibition abrogated GnRH stimulation of FSHβ expression at both high and low pulse frequencies, suggesting that MEK signaling pathways have additional effects on GnRH regulation of FSHβ, beyond those mediated by ICER. Taken together, we conclude that the signaling pathways mediating GnRH activation of CREB and ICER are distinct. The PKA pathway mediates GnRH-stimulated CREB phosphorylation, whereas GnRH induction of ICER occurs via MAPK pathways, contributing to the decoding of the pulsatile GnRH to regulate FSHβ expression.

DOI: 10.1530/endoabs.34.OC5.4

OC5.5

Spatio-temporal analysis of prolactin gene transcription dynamics reveals short range co-ordination of lactotroph transcription activity in pituitary tissue

Karen Featherstone[1], Kirsty Hey[2], Hiroshi Momiji[2], Anne McNamara[1], Amanda Patist[1], David Spiller[1], Helen Christian[3], Alan McNeilly[4], John Mullins[4], Barbel Finkenstadt[2], David Rand[2], Michael White[1] & Julian Davis[1]
[1]University of Manchester, Manchester, UK; [2]University of Warwick, Coventry, UK; [3]University of Oxford, Oxford, UK; [4]University of Edinburgh, Edinburgh, UK.

Mammalian genes display pulsatile transcription dynamics with bursts of expression occurring with variable duration and frequency. Using the human prolactin gene as a model of tissue specific gene regulation, we have characterised the transcription dynamics of this gene in cell lines, primary cells and pituitary tissue slices. Our data indicate that the tissue environment may have an important influence on cellular transcription activity. Cultures of cells from enzymatically dispersed tissue show greater transcriptional pulsatility than cells maintained in their native tissue environment. Temporal transcription patterns of the prolactin gene also change during pituitary development with transient pulses of expression replaced by more stable expression patterns as the pituitary develops.
We have performed spatio-temporal analyses of prolactin gene transcription dynamics in pituitary tissue to assess the nature of tissue influence on gene activity. In adult pituitary tissue, cells display a co-ordinated increase in expression over the first 24 h of culture, potentially due to the removal of the pituitary from the inhibitory influence of hypothalamic dopamine. Patterns of transcription activity from individual cells showed increased correlation over short, one to two cell distances, suggesting that local cellular communication affects transcription activity. Consistent with a cell signalling influence on transcription, limited trypsin digestion, which maintains cells in a tissue structure whilst facilitating the degradation of extracellular proteins, abolished the local increase in correlated activity. In developing pituitary tissue, where lactotroph cell density is reduced, correlation of transcription activity was independent of cellular distance, suggesting that cell communication, if established, is incapable of influencing transcription activity. We are currently investigating whether gap junction mediated transfer of small signalling molecules directly between cells mediates the short range co-ordination of cellular activity that we have detected.

DOI: 10.1530/endoabs.34.OC5.5

Pituitary

OC5.6

A GH antagonist fusion with GH binding protein is biologically active, shows delayed clearance and inhibits growth in a rabbit model

Ian Wilkinson, Michael Phipps, Jon Sayers, Peter Artymiuk & Richard Ross
Sheffield University, South Yorkshire, UK.

Background
Acromegaly is associated with increased morbidity and mortality, however currect medical treatment controls the disease in $<60\%$ of patients. Pegvisomant, a pegylated GH antagonist, controls the disease in over 95% of cases, but is not cost effective as it requires high dose daily injections and has side-effects. We have developed a technology for generating a long acting potent GH antagonist.
Hypothesis
That a GH antagonist fused to GH binding protein will generate a potent long acting antagonist.
Methods
Fusion proteins were purified from CHO cells and tested in a bioassay. Pharmacokinetic studies were conducted in rats and pharmacodynamic studies in growing New Zealand white rabbits. Full ethics approval was obtained for all experiments.
Results
GH antagonist fusions were cloned, expressed and purified. Given at 1 nmol/kg to 6 rats the antagonist showed delayed clearance with plasma half-life of 21.0 h compared to GH at 1.2 h. A single sc dose of GH antagonist at 0.5, 1.0 and 2.0 mg/kg resulted in decreased weight gain over 12 days compared to vehicle control and the decrease in weight gain was greater than that seen after Pegvisomant given as 5 separate injections of 3 mg/kg on days 1, 2, 6, 7 and 8 days (see table).
Conclusions
A fusion of GH antagonist to GH binding protein shows antagonist bioactivity in vitro, delayed clearance in vivo and inhibits growth in rabbits. Based on these observations a GH antagonist fusion has the potential for a potent once weekly subcutaneous therapy for patients with acromegaly.

Table 1

		GH antagonist fusion single dose s.c. at day zero			
Rabbits $n=3$ per group weight gain over 12 days	Vehicle	0.5 mg/kg	1 mg/kg	2 mg/kg	**Pegvisomant** (5× doses at 3 mg/kg)
Mean % weight gain	24.3	17.0	15.5	15.0	17.2

DOI: 10.1530/endoabs.34.OC5.6

Clinical

OC6.1

Adverse outcome in glucocorticoid induced adrenal suppression; an analysis of short synacthen tests in 2782 patients

Matthew Chapman[1], Nicola Argese[2], Dhanasekaran Mani[3], Vijay Dabhi[3], Christopher Boot[4], Rachel Crowley[1], Paul Stewart[5] & Jeremy Tomlinson[1]
[1]School of Clinical and Experimental Medicine, Institute of Biomedical Research, Centre for Endocrinology, Diabetes and Metabolism, University of Birmingham, Birmingham, UK; [2]Faculty of Medicine and Psychology, Endocrinology, Sant'Andrea Hospital, 'Sapienza' University of Rome, Rome, Italy; [3]Health Informatics Department, Queen Elizabeth Hospital, University Hospitals Birmingham, NHS Foundation Trust, Birmingham, UK; [4]Regional Endocrine Laboratory, Queen Elizabeth Hospital, University Hospitals Birmingham, NHS Foundation Trust, Birmingham, UK; [5]School of Medicine, University of Leeds, Leeds, UK.

2–3% of the UK population are prescribed glucocorticoid (GC) therapy and their adverse effects contribute to a significant health burden. Suppression of endogenous GC secretion is a recognized complication of therapy, but the magnitude of the problem, together with its clinical consequences have not been determined. We conducted a retrospective study across all specialties in a large secondary–tertiary care center identifying 2782 patients who underwent 3666 250 μg short Synacthen tests (SST) between 2008–2013. Patients were grouped according to indication and clinical and biochemical assessments made prior to SST were analyzed; a 30-min cortisol of ≥ 550 nmol/l was considered indicative of adequate adrenal reserve.
497 (17.9%) patients failed the SST; failure rates were highest in those patients with underlying adrenal disease (60.8%). 693 patients had pituitary disease and 21.5% ($n=142$) failed the SST. 408 patients were taking oral, topical, intranasal or inhaled GC therapy (excluding those with pituitary, adrenal and central nervous system pathology) and 32.8% ($n=134$) of these patients failed. In this group, 30-min cortisol response was positively associated with systolic blood pressure ($P<0.05$), and those patients with the highest cortisol response had fewest elective hospital admissions in the preceding 2 years (0.8 ± 0.2 vs 0.3 ± 0.1, $P<0.05$). In those patients taking inhaled corticosteroids (those with oral co-administration were excluded), Fluticasone treatment was associated with the highest SST failure rates (26.1%). However, in patients taking either inhaled fluticasone or beclometasone, there was a dose-dependent effect upon the cortisol response across the SST; those patients on the highest doses having both decreased basal and 30-min cortisol values (e.g. Fluticasone 30-min cortisol, <400 vs >400 μg/day, 880 ± 72 vs 737 ± 34 nmol/l, $P<0.05$).
Suppression of adrenal reserve due to GC administration (oral, topical or inhaled) is both common and associated with adverse outcome. Future prospective studies need to define appropriate interventional strategies and determine their impact upon clinical outcome.

DOI: 10.1530/endoabs.34.OC6.1

OC6.2

Localising parathyroid adenomas: which imaging modality is best? Pre-operative localisation studies in patients with primary hyperparathyroidism: a large audit in a London tertiary centre

Danielle Lewis, J Hubbard, M Moonim, D Dasgupta, S Thomas, J K Powrie, P V Carroll & B M McGowan
St Thomas' Hospital, London, UK.

Parathyroidectomy is the only definitive cure for primary hyperparathyroidism (PHPT). The standard for pre-operative localisation of parathyroid pathology at our institution is both a (99m)Tc-sestamibi SPECT/CT (sestamibi) and neck ultrasound scan (USS). The aim of this audit was to assess the accuracy of this standard pre-operative imaging.

Methods
Retrospective data was gathered from all parathyroidectomies performed at St Thomas' Hospital between 2008 and 2011.
Results
164 parathyroid operations were identified with complete data available for 140 patients (included in this report). Of these, 72.9% were female, age 17–87 years (mean 56). Histology identified 111 (79.3%) adenomas, 21 (15%) hyperplasia, 2 (1.4%) adenoma plus hyperplasia and 6 (5%) 'other pathology'.
Sestamibi scans were performed in 134 patients (95.7%) with accurate localisation in 98 (73.1%). USS were performed in 115 patients (82.1%) with accurate localisation in 63 (54.8%). Of the 109 patients (77.9%) who had both sestamibi and USS; both scans correctly identified an adenoma in 51 (48.8%) cases: in 25 cases (22.9%) only the sestamibi correctly identified the lesion, in 10 (9.2%) cases only the USS correctly identified the lesion. Neither scan correctly identified the lesion in 23 (21.1%) cases.
In 140 patients, sestamibi and/or USS correctly identified the location of parathyroid pathology in 108 patients (77.1%). Therefore, pre-operative imaging was not helpful in 22.9% of patients.
Discussion
Pre-operative sestamibi and/or USS accurately located parathyroid pathology in over 75% of cases. Sestamibi was requested more often and was more accurate at pre-operative localisation vs USS. USS identified parathyroid pathology in 10 (9.2%) of patients in whom the sestamibi was non-diagnostic. USS should be reserved for those patients with negative sestamibi rather than used routinely in pre-operative localisation of parathyroid pathology.

DOI: 10.1530/endoabs.34.OC6.2

OC6.3

Sirolimus therapy for a patient with segmental overgrowth due to a mosaic activating mutation in phosphatidylinositol-3-kinase

Victoria Parker[1], Susan Huson[2], Iona Isaac[1], Julie Harris[1], Felicity Payne[3], Marjorie Lindhurst[4], Ines Barroso[3], Leslie Biesecker[4] & Robert Semple[1]
[1]Wellcome Trust MRC Institute of Metabolic Science, Cambridge, UK; [2]Manchester Centre for Genomic Medicine, Manchester, UK; [3]The Wellcome Trust Sanger Institute, Hinxton, UK; [4]The National Human Genome Research Institute, Bethesda, Maryland, USA.

Background
Phosphatidylinositol-3-kinase (PI3K) is a critical mediator of insulin action, and influences cellular growth, survival and metabolism. Recently, somatic gain-of-function mutations in the p110α catalytic subunit of PI3K (PIK3CA) have been found in a spectrum of overgrowth disorders, outlining the possibility of treatment with inhibitors of the PI3K–AKT–mTOR pathway.
Methods/Results
A 37-year-old female, presented with life-long, massive, progressive overgrowth of her legs in association with a lean, normal sized upper body. There was no history of malignancy, nor was their evidence for metabolic disturbance on biochemical profiling. Magnetic resonance imaging and histologic analysis demonstrated overgrowth of fibroadipose tissue in her legs, with fatty infiltration of muscles. We performed whole exome sequencing on DNA extracted from her legs and arms, and identified a heterozygous mis-sense mutation p.*PIK3CA*. His1047Leu, exclusively in the legs. Using mass spectrometry we found elevated basal PIP_3 levels in leg derived dermal fibroblasts, and concomitant hyperactivation of key growth signals, AKT and p70S6K, and this was reversed by chronic exposure to the mTOR inhibitor everolimus.
Sirolimus therapy was initiated in the patient on clinical grounds due to continued growth and compromised mobility. Treatment was well tolerated, and at 1 year a 12% reduction in fat mass from the legs was recorded by dual-energy X-ray absorptiometry scanning.
Discussion
A mosaic activating *PIK3CA* mutation has caused a phenotype of progressive overgrowth of mainly adipose tissue in the legs of this patient. Metabolic disturbance was not prominent, and we speculate that this is because the liver is unaffected. Proof-of-concept studies in dermal fibroblasts suggest mTOR inhibitors could be an effective treatment strategy, and our index patient has made encouraging progress on sirolimus. These findings will, however, need to be substantiated by a future randomised controlled trial, and p110α inhibitors, currently in phase development, remain an attractive prospect for the future.

DOI: 10.1530/endoabs.34.OC6.3

OC6.4

Reduced cognitive performance and altered white matter microstructure in young insulin-resistant women with polycystic ovary syndrome

Maneesh Udiawar[1], Rok Berlot[2], Michael O'Sullivan[2] & Aled Rees[1]
[1]School of Medicine, Centre for Diabetes and Endocrine Sciences, Cardiff University, Cardiff, Wales, UK; [2]Institute of Psychiatry, Academic Neuroscience Centre, London, UK.

Background
Metabolic disorders such as type 2 diabetes are risk factors for the development of dementia. In addition to vascular dysfunction, insulin resistance may be important since altered insulin sensitivity is associated with changes in neurogenesis. Polycystic ovary syndrome (PCOS) is a disorder characterised by insulin resistance. Little is known about the impact of metabolic abnormalities on brain structure and function in younger adults.
Objective
To examine whether PCOS is associated with microstructural abnormalities and to relate these to cognitive functioning.
Methods
women with PCOS (age 31 ± 6 years, BMI 30 ± 6 kg/m^2) and 19 control subjects (age 31 ± 7 years, BMI 29 ± 6 kg/m^2) underwent diffusion tensor MRI and detailed cognitive assessment. Diffusion tensor imaging measures, including fractional anisotropy (FA) and mean diffusivity (MD) were compared between groups and related to cognitive performance. We analysed FA, MD and axial diffusivity (AD) values by using a tract based spatial statistics (TBSS) technique (whole brain analysis). Tractography was also performed to reconstruct individual temporal lobe white matter tracts.
Results
Subjects with PCOS had higher testosterone (1.56 ± 0.6 (PCOS), 0.9 ± 0.6 nmol/l (controls); $P=0.01$) and insulin response to glucose challenge (IAUC 93 151 $\pm 42\,694$ (PCOS), $61\,933 \pm 29\,614$ (controls); $P=0.04$). Despite similar educational achievement (NART IQ 122, $P=0.35$) subjects with PCOS performed less well on a summary measure of cognitive performance (principal components analysis, $t=2.9$, $P=0.007$). TBSS showed areas of decreased AD in PCOS throughout the white matter skeleton. The genu of the corpus callosum and right cingulum also showed decreased MD and altered tissue volume fraction in PCOS ($P<0.05$).
Conclusions
Subjects with PCOS performed less well than controls in a measure of executive function and episodic memory. This is not attributable to BMI or premorbid intelligence. Our data suggest that alterations in brain structure, and subtle deficits in cognition, emerge at a young age in insulin-resistant subjects.

DOI: 10.1530/endoabs.34.OC6.4

OC6.5

Effectiveness of metyrapone in 195 patients with Cushing's syndrome

Eleni Daniel[1], Simon Aylwin[2], Omar Mustafa[2], Steve Ball[3,4], Atif Munir[4], Kristien Boelaert[5], Vasileios Chortis[5], Daniel J Cuthbertson[6], Christina Daousi[6], Surya Rajeev[7], Julian Davis[8], Kelly Cheer[9], William Drake[10], Kirun Gunganah[10], Ashley Grossman[11,13], Mark Gurnell[12], Andrew Powlson[12], Niki Karavitaki[11,13], Isabel Huquet[13], Tara Kearney[14], Kumar Mohit[14], Karim Meeran[15], Neil Hill[15], Aled Rees[16], Peter J Trainer[17], Andrew J Lansdown[16], Anna-Elisabeth H Minder[17] & John Newell-Price[1]
[1]The Medical School, University of Sheffield, Sheffield, UK; [2]King's College Hospital NHS Foundation Trust, London, UK; [3]The Medical School, Newcastle University, Newcastle, UK; [4]Royal Victoria Infirmary, Newcastle, UK; [5]College of Medical and Dental Sciences, Centre for Endocrinology, Diabetes and Metabolism, University of Birmingham, Birmingham, UK; [6]Department of Obesity and Endocrinology, University of Liverpool, Liverpool, UK; [7]Aintree University Hospital, Liverpool, UK; [8]Centre for Endocrinology and Diabetes, University of Manchester, Manchester, UK; [9]Manchester Royal Infirmary, Manchester, UK; [10]Department of Endocrinology, St Bartholomew's Hospital, London, UK; [11]University of Oxford, Oxford, UK; [12]School of Clinical Medicine, University of Cambridge, Cambridge, UK; [13]Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK; [14]Salford Royal NHS Foundation Trust, Salford, UK; [15]Imperial College, London, UK; [16]School of Medicine, Cardiff University, Cardiff, UK; [17]The Christie NHS Foundation Trust, Manchester, UK.

Background
Metyrapone is widely used in the UK for the control of cortisol excess in Cushing's syndrome, but its use is not standardised. There are a few reports published on metyrapone use, mostly containing small patient numbers.

Method
A retrospective survey was conducted across 13 tertiary centres. Using a standardised proforma, extensive data including monitoring and safety information were collected for patients with Cushing's syndrome on metyrapone therapy between 1997 and 2013.
Results
195 patients received metyrapone (160 on monotherapy). Average age was 49.6 ± 15.7 years. Aetiology of Cushing's syndrome: pituitary-dependent disease (CD, 59% (macroadenoma 32% of CD)), ectopic ACTH syndrome (EAS, 18.9%), adrenocortical carcinoma (ACC, 14.9%) and adrenal adenoma (AA, 7.1%): 73.3% received metyrapone prior to surgery and 12.8% had cortisol-lowering therapy alone. Dose-titration was used in 81% of patients, whereas 19% had block-and-replace. The average starting dose was 1055 mg; median doses were 750 mg for CD and AA, 1000 mg for EAS, and 1500 mg for ACC. The preferred monitoring method was by cortisol day-curves, followed by 0900 h cortisol and urinary free cortisol. Hypokalaemia on therapy was actively managed, with potassium levels increasing during treatment (3.95 vs 3.66 mmol/l, $P < 0.0001$). The mean treatment duration was 8 months, 81.4% achieving eucortisolaemia on varying doses: CD 1390 mg, EAS 1900 mg, AA 1080 mg, and ACC 1500 mg. 25.3% of patients developed side effects; most commonly gastrointestinal upset and hypoadrenalism. 88% of adverse events were managed as outpatients; 36% of patients treated for more than one month had ≤2 monitoring assessments and insufficient dose titration.
Conclusion
This is the largest report of metyrapone use. Metyrapone was effective in achieving eucortisolaemia in over 80% of patients, with a satisfactory safety profile. A variety of monitoring regimens were used, but greater standardisation of practice and more active dose titration is needed.

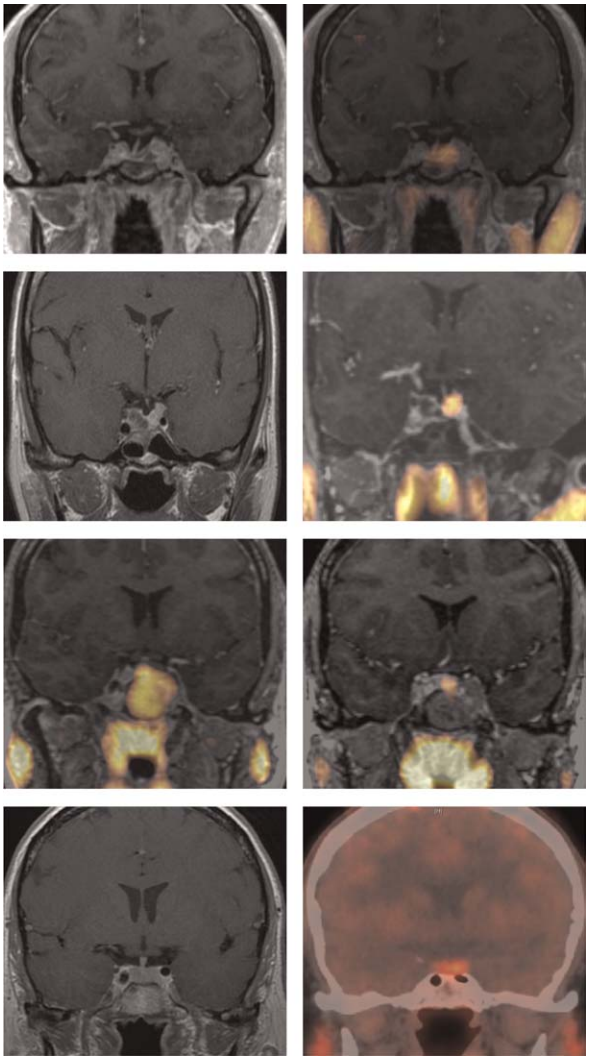
DOI: 10.1530/endoabs.34.OC6.5

OC6.6

^{11}C-methionine PET–CT co-registered with volume MRI: a novel adjunctive imaging modality to aid diagnosis and management in patients with pituitary adenomas

Olympia Koulouri[1,2], Narayanan Kandasamy[1], Andrew Powlson[1,2], Carla Moran[1,2], Heok Cheow[2], Nagui Antoun[2], Miles Levy[3], Andrew Hoole[2], Krishna Chatterjee[1,2], Neil Donnelly[2], Richard Mannion[2], Neil Burnet[1,2], John Pickard[1,2] & Mark Gurnell[1,2]
[1]University of Cambridge, Cambridge, UK; [2]Addenbrooke's Hospital, Cambridge, UK; [3]University Hospitals of Leicester, Leicester, UK.

Although MRI remains the investigation of choice for pituitary imaging, it does not provide information about 'functionality' of lesions (e.g. residual adenoma vs post-surgical scar tissue) and cannot reliably identify all microadenomas.
We hypothesised that i) imaging with the PET ligand ^{11}C-methionine, which is taken up at sites of peptide/protein synthesis, would permit more reliable identification of functioning pituitary adenoma and ii) co-registration of PET–CT with volume MRI (MetPETCT–MRI) would yield more accurate anatomical localisation of ^{11}C-methionine uptake.
80 scans were performed in our centre between 2010 and 2013. MetPETCT–MRI was found to provide additional useful information in the following scenarios:
(A) Distinguishing residual functioning tumour from inactive or scar tissue post pituitary surgery (Figs 1 and 2) or medical therapy (e.g. cabergoline in prolactinoma) ($n = 29$)
(B) Demonstrating medical therapeutic effects through significant reduction of tracer uptake post- compared to pre-treatment (Fig. 3) ($n = 15$).



DOI: 10.1530/endoabs.34.OC6.6

Poster Presentations

Bone

P1

Primary hyperparathyroidism in pregnancy

Jehangir Abbas & Rudy Bilous
The James Cook University Hospital, Middlesbrough, UK.

Introduction
We submit a rare presentation of primary hyperparathyroidism in pregnancy posing complex management difficulties.
Case report
A 32-year-old Asian woman presented during third trimester of her pregnancy with symptoms of lethargy, leg pain, and weakness. Routine investigations revealed an elevated calcium level (3.83 mM) and PTH level of 231.1 ng/l. She was treated with i.v. fluids and pamidronate. An ultrasound of her thyroid gland showed a large superior left parathyroid adenoma measuring 1.9 $\times$1.5 $\times$ 4.1 cm. She had a background history of a still birth at 28+6 weeks which was complicated by chickenpox followed by several miscarriages (9) in the first trimester. She was investigated in two different tertiary centres and a possible diagnosis of antiphospholipid syndrome was made due to a borderline positive lupus anticoagulant and anti cardiolipin antibody. She also had a history of severe vitamin D deficiency (level $<$10 nmol/l) with borderline high calcium (2.64 mM) and very high PTH 386 ng/l 5-yearly previously.She was treated with alpha calcidol and refered to local endocrine team but follow- up was lost because she moved. Owing to this background an urgent caesarean section was performed and healthy baby was delivered at 33 weeks and 4 days. After delivery she was started on cinacalcet to control her calcium levels. Elective para thyroidectomy was performed and now her calcium level and PTH are currently normal. Parathyroid gland biopsy results were in keeping with either adenoma or hyperplasia.
Discussion
Primary hyperparathyroidism during pregnancy poses significant risks to the mother and the foetus. Fortunately, prompt diagnosis and effective management can improve outcomes for both. There is controversy regarding appropriate management of these patients, especially late in gestation. The objective of this case report, therefore, is to review the literature and to propose an evidence-based approach to managing these patients. The prevalence of primary hyperparathyroidism in the general population is 0.15%. In addition to many constitutional symptoms, maternal complications include nephrolithiasis, bone disease, pancreatitis, hyperemesis, muscle weakness, mental status changes, and hypercalcemic crisis. Reported fetal complications include intrauterine growth retardation, low birth weight, preterm delivery, intrauterine fetal demise, postpartum neonatal tetany, and permanent hypoparathyroidism.

DOI: 10.1530/endoabs.34.P1

P2

Primary hyperparathyroidism in pregnancy presenting as hyperemesis

Rashmi Manjunatha[1], Jyothi Nippani[2], Deborah Markham[3] & Rajni Mahto[1]
[1]Department of Diabetes and Endocrinology, Warwick Hospital, Warwick, UK; [2]Department of Obstetrics and Gynaecology, Warwick Hospital, Warwick, UK; [3]Department of ENT, Warwick Hospital, Warwick, UK.

Primary hyperparathyroidism in pregnancy can be associated with serious complications. Maternal complications include, increased risk of pre-eclampsia/eclampsia, miscarriage, arrythmia during labour/delivery, and still/premature birth. Neonatal complications include risk of permanent hypoparathyroidism, tetany, seizures, hypotonia, low birth, weight and respiratory distress. Early part of second trimesters is the best time to operate, as risks of surgery and anaesthesia are minimal, foetal parathyroids are formed during second and third trimester and hypercalcemia in third trimester may lead to hypertension/pre-eclampsia. We present a case of primary hyperparathyroidism presenting during pregnancy as hyperemesis.
A 22-year-old primiparous lady with poorly controlled type 2 diabetes, hypertension, morbid obesity (BMI 39.4) was admitted at 12 weeks gestation with intractable vomiting. She had similar admissions earlier in this pregnancy with 'hyperemesis'. She was found to have a biochemical picture of primary hyperparathyroidism, with raised calcium of 3.16 mmol/l and elevated PTH of 17.9 pmol/l. TSH was normal (1.62 mU/l). Ultrasound of parathyroid revealed a 9.5 mm well defined soft tissue nodule in the right side, raising a probability of parathyroid adenoma. During second trimester, she underwent an open exploration of the neck and removal of a single slightly large parathyroid gland from her right neck. Although the parathyroid hormone levels dropped initially, it plateaued at $\sim$ 11 pmol/l. Six days later, she underwent re-exploration and removal of left parathyroid gland which was retro-tracheal in position. Intra-operative parathyroid hormone estimations showed a satisfactory drop in PTH level to 1.1 pmol/l. Intra-operative frozen section confirmed an adenoma. Post-operatively, she was established on α-calcidol. She delivered a healthy baby at term.
This case highlights the importance of checking calcium in pregnant women presenting with intractable vomiting. Intra-operative PTH measurements could be useful during difficult operations. Timely multidisciplinary approach in the management of such a patient is crucial for best maternal and foetal outcomes.

DOI: 10.1530/endoabs.34.P2

P3

The utility of bone turnover markers in Paget's disease of bone

Mark J Hannon[1], Isolda M Frizelle[1], Mark Kilbane[2] & Malachi J McKenna[1,2]
[1]Department of Endocrinology, St Michael's Hospital Dun Laoghaire, Co. Dublin, Ireland; [2]St Vincent's University Hospital Metabolism Laboratory, Dublin, Ireland.

Bone turnover markers (BTMs) may have a role in assessing bone turnover and response to intervention in Paget's disease. We examined the clinical utility of bone turnover markers at diagnosis through analysis of our database of patients with radiographically confirmed Paget's disease.
We identified 36 patients (20 men); mean age at diagnosis was 71.6 years (range 54–84). Radionuclide imaging identified that 64% had polyostotic disease, with no gender difference. At diagnosis, all patients had estimation of ionised calcium, total alkaline phosphatase (total ALP), parathyroid hormone (PTH), and 25-hydroxyvitamin D (25OHD). The following BTMs were measured in serum in the fasting state: bone-specific alkaline phosphatase (bone ALP), procollagen type I N-propeptide (PINP), intact osteocalcin (OC(1–49)), and C-terminal cross-linking telopeptide (CTX). Fasting spot urine was collected for measurement of N-terminal cross-linking telopeptide (NTX).
Serum 25OHD was $<$50 nmol/l in 36%; 2/36 (6%) had raised PTH; none were hypercalcaemic. The prevalence of abnormal BTMs was as follows: total ALP (67%); bone ALP (67%); PINP (36%); OC(1–49) (36%); CTX (31%); and NTX (44%). Total ALP correlated significantly with bone ALP ($r=0.48$, $P=0.016$), with OC(1–49) ($r=0.47$, $P=0.03$), with PINP ($r=0.73$, $P<0.0001$), and with NTX ($r=0.59$, $P=0.006$) but not with CTX. Total ALP was significantly higher in those with polyostotic disease at diagnosis ($P=0.019$) as was NTX ($P=0.02$) but not other BTMs. Fourteen patients were screened for the Q3STM1 mutation; two were positive. One of these patients had the only raised osteocalcin identified; otherwise there were no differences between these and the Q3STM1-negative cohort.
While BTMs are not needed for the diagnosis of Paget's disease, they are helpful is quantifying the degree of activity and in assessing the response to bisphosphonate therapy. Total ALP has similar utility to the newer BTMs, and would be a cheaper substitute assuming that the patient did not have concomitant hepatobilary disease.

DOI: 10.1530/endoabs.34.P3

P4

An audit of the clinical indications for initiation of Denosumab as a treatment for Osteoporosis in a secondary care clinic

Angela Collie[1], Khun Phyomaung[1], Menai Shahim[1], Stephen Gallacher[1], Andrew Gallagher[2], Fergus McLean[1], John Ahukannah[1] & John Hinnie[2]
[1]Southern General Hospital, Glasgow, UK; [2]Victoria Infirmary Hospital, Glasgow, UK.

Objective
Denosumab is a highly specific MAB, which binds to RANK Ligand thus inhibiting osteoclast formation, function and survival[1].
It's efficacy in the treatment of osteoporosis was demonstrated in the FREEDOM trial which was an international, randomised, placebo controlled trial involving 7686 post menopausal women. The trial demonstrated a reduction in risk for vertebral fractures (68%), non vertebral fractures (20%), and hip fractures (40%)[2]. Denosumab was approved in 2010 for the treatment of post menopausal women with osteoporosis. This audit looks at clinical indications for which Denosumab is being prescribed in a secondary care mineral metabolism clinic.
Methods
Eighty-five patients attending a mineral metabolism clinic in secondary care were prescribed Denosumab between 12/01/12 (the date the first use of Denosumab at the clinic) and 09/10/13.The reason for prescription was categorised as i) poor outcome with previous treatment, ii) bisphosphonate intolerance, iii) eGFR $<$35 ml/min, iv) fracture while on 'bishosphonate holiday', v) patients with learning difficulties, and vi) other.
Results
The total number of patients was 85, of whom 78 were female and seven males. The mean age was 74 with an age range 43–90.The distribution between the categories was: Poor outcome with previous treatment 46%, bishosphonate

intolerance 20%, eGFR < 35 ml/min 18%, fracture while on 'bisphosphonate holiday' 6%, patients with learning difficulties 3%, and other 7%.
Conclusion
This audit illustrates the various ways in which Denosumab is being used in a secondary care mineral metabolism clinic.
References
1. Boyle WJ *et al*. *Nature* 2003 **423** 337–342.
2. Cummings SR *et al*. *New Eng J Med* 2009 **361** 756–764.
DOI: 10.1530/endoabs.34.P4

P5

Value of sequential 25-hydroxyvitamin D measurements prior to denosumab and zoledronic acid treatment
Pushpa Singh & Neil Gittoes
University Hospital Birmingham, Birmingham, UK.

Patients receiving parenteral forms of treatment for osteoporosis (e.g. denosumab (DMAB) and zoledronic acid (ZOL)) should be vitamin D replete prior to administration, to minimise the risk of hypocalcaemia. Most patients receiving DMAB or ZOL take supplemental (calcium and) vitamin D but it is widely accepted that adherence to calcium and vitamin D is poor. Frequency of testing 25-hydoxyvitamin D (25OHD), calcium and renal function varies widely between centres in the UK. In recent times, our local protocol has been to check these variables ~ 2 months prior to each injection/infusion and only proceed to treatment when the 25OHD is ≥50 nmol/l. This audit was established to determine the value of this intensive monitoring protocol and whether it informs practice.
Case records of 240 patients who received DMAB (60) or ZOL (180) were reviewed. In the DMAB group, prior to first treatment, 27.3% (12) had 25OHD <50 nmol/l, (7% were <30) and 58% were already taking calcium/vitamin D supplements. In the ZOL group, 40% had 25OHD <50 nmol/l (15% were <30 nmol/l) prior to the 1st infusion; 33% were already taking calcium/vitamin D supplements. Prior to administration of the second dose of ZOL/DMAB 17–24% still had 25OHD <50 nmol/l. Prior to the third and fourth doses of ZOL/DMAB, 26–12 and 20-0% respectively had 25OHD <50 nmol/l, despite ongoing Ca/D supplementation. In those with low 25OHD, adjustments to vitamin D intake were made so as to achieve the target 25OHD concentration of 50 nmol/l.
Despite prescription of Ca/D, 25OHD levels are frequently suboptimal prior to drug administration. The proportion of those with 25OHD <50 nmol/l falls with subsequent dosing and time but even after the 3rd dose of ZOL, 20% patients had 25OHD <50 nmol/l. The data justifies an aggressive approach to pre-treatment testing of 25OHD. We believe this is important for patient safety and to mitigate risks of post-treatment hypocalcaemia.
DOI: 10.1530/endoabs.34.P5

P6

Impact of vitamin D replacement in patients with primary hyperparathyroidism and co-existing vitamin D deficiency
Gautam Das, Vinay Eligar, Jyothish Govindan & Stephen Davies
University hospital of Wales, cardiff, UK.

Introduction
Vitamin D deficiency/insufficiency in patients with primary hyperparathyroidism may be associated with more severe and progressive disease. In such patients there is higher levels of parathormone and markers of bone turnover, large parathyroid adenomas and more frequent fractures than vitamin D replete patients.
Aims and objectives
To determine whether vitamin D repletion of patients with PHPT and co-existing vitamin D insufficiency or deficiency has any adverse effects on their biochemical parameters.
Methods
A retrospective analysis of data for one year (2012) from the hospital database for patients who had biochemical evidence of raised PTH and had a vitamin D levels checked at presentation ($n=111$). Patients with satisfactory levels of 25(OH) D levels at onset ($n=37$), those with insufficient data at beginning or follow up and those who were not replaced with vitamin D ($n=35$) and those who had normocalcemia with raised PTH ($n=23$) due to secondary hyperparathyroidism were excluded. 16 patients were included for the study (with hypercalcemia >2.62 mmol/l and raised PTH levels) and were considered to have insufficient levels of vitamin D if their 25(OH) D were between 10–20 μg/l ($n=9$) and deficient in vitamin D if the 25(OH) D levels were < 10 μg/l ($n=7$). Patients were treated with either different dosage of colecalciferol (Fultium-D3) (800IU, 1600IU, and 3200IU) alone or a combination of calcium carbonate and colecalciferol (500 mg/400IU).
Results
The mean age of the combined study group was 68.25 ± 11.48 years and the average follow up period was 9.81 ± 6.49 months. After replacement with colecalciferol, 25 (OH) D levels improved significantly (11.63 ± 4.31 vs 28.67 ± 11.91 μg/l, $P<0.0001$) together with a fall in adjusted calcium (2.75 ± 0.12 to 2.65 ± 0.15 mmol/l, $P=0.02$). However, PTH levels remained unchanged (18.57 ± 8.12 to 17.96 ± 12.47, $P=0.43$).
Conclusion
In contrast to what would be perceived, our data reveal that vitamin D repletion in patients with PHPT appears to modestly reduce calcium concentrations. The mechanism for this is unclear but as with the increase in 25 (OH) D attenuation of secondary hyperparathyroidism may assist. The study also reveals that vitamin D3 can be safely used to replace deficiency/insufficiency in patients with hypercalcemic PHPT.
DOI: 10.1530/endoabs.34.P6

P7

Monitoring the bisphosphonate treatment holiday
Clare Mumby, Shazli Azmi, Peter Selby, Katharine Hayden & Judith Adams
Manchester Royal Infirmary, Manchester, UK.

There is concern about the effects of long-term bisphosphonate use. A 'treatment holiday' is now considered after 5 years but there is no evidence as to the best way in which to monitor this.
Aim
To look at the effect that a 'treatment holiday' has on the bone turnover marker, amino-terminal propeptide (P1NP) and bone mineral density (BMD).
Methods
A retrospective case note review of 55 patients currently undergoing a bisphosphonate treatment holiday. P1NP and BMD were recorded at baseline and at intervals during the treatment holiday. The BMD was recorded as a percentage change to determine clinical significance (±3%).
Results
There were 55 subjects aged 68.9±1.7 who had received treatment with a bisphosphonate for 87.2±4.5 months prior to stopping. The P1NP when the bisphosphonate was stopped was 18.8 μg/l±1.5. There was a significant increase in P1NP at 1 year (31.1±1.8, $P=0.000$) but no significant change at 2 years (32.8±1.8) and 3 years (32.1±3.5). Hip BMD and Spine BMB remained clinically stable at 24 months with a percentage change at the hip of −1.1±0.5 and the spine 0.1±0.8.
11 patients has a significant (>3%) bone loss at the hip at 24 months. These patients had a rise in P1NP of 21.7±4.3 from baseline at 12 months compared to a rise of 13.9±2.2 in those with no significant bone loss. There was a significant correlation between the level of increase of P1NP at 12 months and the percentage change in BMD at total hip ($r=-0.35$, $P=0.04$).
Conclusion
After an initial rise in P1NP after stopping the bisphosphonate this then appears to remains stable for at least 3 years. On average bone mineral density remains clinically stable for at least 24 months. A greater rise in P1NP at 1 year may predict the level of bone loss at 24 months.
DOI: 10.1530/endoabs.34.P7

P8

Liraglutide, a glucagon-like peptide-1 receptor agonist, improves bone mass and architecture in ovariectomised mice
Marie Pereira, Jeshmi Jeyabalan, Camilla Sofie Jørgensen, Mark Cleasby, Mark Hopkinson & Chantal Chenu
Royal Veterinary College, London, UK.

The increased incidence of type 2 diabetes mellitus (T2DM) among the aged is associated with an impaired skeletal structure and a higher prevalence for bone fractures. Besides, anti-diabetic therapies can also negatively affect bone mass. In this study, we tested the skeletal effects of chronic administration of two glucagon-like peptide receptor (GLP-1R) agonists and examined the expression of GLP-1R in bone tissue and cells. Twelve week-old female C57Bl/6N mice were ovariectomised (OVX) to induce bone loss. Four weeks after OVX, mice were treated with either: Liraglutide (LIR) 0.3 mg/kg per day, Exenatide (Ex-4)

10 μg/kg per day or saline for 4 weeks ($n=10$/group). Cortical and trabecular bone architecture was analysed in tibias by micro-CT and serum samples collected for evaluation of sclerostin levels, an inhibitor of bone formation. RNA was extracted from control femora and osteoblastic cell lines to analyse the presence of GLP1R using RT-PCR. Our results show that LIR significantly increases bone mass compared to controls manifested by higher trabecular bone volume percent ($+61\%$, $P<0.01$), trabecular number ($+46\%$, $P<0.05$), while trabecular pattern factor (-15%, $P<0.05$) and structure model index (SMI) (-9%, $P<0.05$) were significantly decreased, reflecting improvement in trabecular bone structure and connectivity. The cortical indexes were not significantly affected by LIR. EX-4 showed a similar tendency for improvement of trabecular mass and architecture, although the effects were non-significant, except for SMI (-9%, $P<0.05$). We could not detect GLP1R in bone, UMR-106 rat osteoblastic and MLO-A5 mouse osteocytic cell lines, suggesting that GLP1R agonists may affect bone indirectly. Serum sclerostin levels were significantly decreased by Ex-4 (-23%, $P<0.05$) but not LIR, suggesting that Ex-4 may decrease the inhibition of bone formation induced by sclerostin. Our data suggest that GLP1R agonists may have beneficial effects on bone and further studies will determine their mechanisms of action.

DOI: 10.1530/endoabs.34.P8

P9

Pre-operative localisation studies in primary hyperparathyroidism: concordance with surgical findings and histology

Edward McKeever[1], Robert Kennedy[2], Stephen Kirk[2], Roy Harper[1], Darren McLaughlin[1], C Mulligan[1], Tom Lynch[3], Clive Majury[4] & CM McHenry[1]
[1]Department of Endocrinology and Diabetes, Ulster Hospital, Dundonald, UK; [2]Department of Breast and Endocrine Surgery, Ulster Hospital, Dundonald, UK; [3]Department of Nuclear Medicine, Belfast City Hospital, Belfast, UK; [4]Department of Radiology, Ulster Hospital, Dundonald, UK.

Successful minimally invasive parathyroidectomy for primary hyperparathyroidism depends on accuracy of pre-operative localisation studies. Ultrasound (US) and sestimibi (SM) scanning remain the imaging modalities of choice reserving MRI, CT and PET for patients who have not been cured by previous explorations or for whom other localization techniques are uninformative or discordant. The aim of this study was to review the accuracy of US and SM in the pre-operative localisation of parathyroid adenomas.

We performed a retrospective review of 51 consecutive patients with a biochemical diagnosis of primary hyperparathyroidism who underwent surgery by one of two Endocrine Surgeons. We compared findings on ultrasound and ^{99m}Tc-sestamibi scintigraphy to histology results.

Of the 51 patients who underwent parathyroid surgery from January 2012 – June 2013, complete data was available for 47 (M:F 12:35; median age 64 years, range 15–81). Primary hyperparathyroidism was confirmed biochemically with pre-operative calcium 2.85 ± 0.17 mmol/l and parathyroid hormone concentration $126.0\pm$ 113.0 pg/ml. Thirty six patients had a solitary parathyroid adenoma, six had parathyroid hyperplasia, one had multiple adenomas and four had inconclusive histological findings. Ultrasound was positive in 29 of 36 (80.6%) adenomas with precise anatomical position found in 22 of the 29 giving a sensitivity, specificity and positive predictive value of 81, 64 and 88%, respectively. Pre-operative ^{99m}Tc-sestamibi scintigraphy correctly identified 21 of 36 (58.3%) adenomas with 58.3% sensitivity, 81.8% specificity and 91.3%. positive predictive value. US findings correlated with SM in 20 patients and were 85% accurate giving sensitivity 81.2%, specificity 100% and positive predictive value 100%.

US and SM scanning show good concordance with histology following parathyroid surgery and when combined provide accurate pre-operative localisation. They should remain the first line to guide minimally invasive parathyroidectomy while other techniques should be reserved for when results are discordant or for those who need re-exploration following failed surgery.

DOI: 10.1530/endoabs.34.P9

P10

Abstract Withdrawn.

DOI: 10.1530/endoabs.34.P10

P11

Unusual cause of hypocalcemia

Lavanya Pelluri, Christian Hariman & Narasimha Murthy
University Hospital Coventry and Warwickshire, Coventry, UK.

Introduction

We present a rare cause of hypoparathyroidism and hypocalcemia and implications of the same during pregnancy.

Case report

A 22-year-old primigravida was referred to the antenatal endocrine clinic with a longstanding history of hypoparathyroidism with hypocalcaemia. She had short stature and extended digits relative to her height. She has been taking alfacalcidol 0.5 μg once a day and adcal D3 tablets and blood tests revealed low normal calcium and PTH levels. Her 20 weeks antenatal scan showed foetal cardiac abnormalities severe enough to consider medical termination of pregnancy. Post mortem examination of the foetus confirmed a baby girl with truncus arteriosus and a ventricular septal defect. Genetic studies of the foetus showed karyotype of 46XX, with a microarray analysis identifying a micro-deletion at 22Q11.21 consistent with DiGeorge/velocardiofacial syndrome. Further genetic tests performed on the patient and spouse confirmed that she was a heterozygous carrier with the same micro-deletion compatible with DiGeorge syndrome and her spouse had no chromosomal abnormalities. A subsequent echocardiogram of the patient demonstrated no significant cardiac abnormalities.

Conclusion

DiGeorge syndrome or 22q11.2 deletion syndrome is a rare disorder caused by micro-deletion of a small piece of chromosome 22 normally manifesting with thymic aplasia (causing immune deficiency), hypocalcemia, hypoparathyroidism, congenital heart defects, and palatal cleft defects. The micro-deletion occurs near the middle of the chromosome at the loci q11.2. This condition should be considered in the differential diagnosis of hypoparathyroidism on its own as it has different levels of penetrance and can have implications when considering pregnancy

DOI: 10.1530/endoabs.34.P11

P12

The influence of gender on the bone health of adolescent patients with hormonal deficiencies

Kerrie Grounds, Pamela Corlett, Aftab Ahmad & Pauline Whittingham
Royal Liverpool University Hospital, Liverpool, UK.

Introduction

Previous audits demonstrated low bone mineral density (BMD) in adolescent patients with hormone deficiencies. We wanted to ascertain if gender had any relationship with the development of low BMD.

Method

A retrospective analysis of 42 transitional clinic patients who underwent DEXA scanning was made using case notes and hospital systems. Follow-up data was gathered as previous audit of 25 transitional patients had shown a significant number to have low BMD.

Results

A total of 42 patient records were accessed, of these 33 patients were male. Mean age for both genders was 21. Age range at baseline was 16–21 years for male and 17–20 years for females. Twenty males (60.6%) and $n=7$ (77.7%) females had low BMD at baseline scans. There were 16 patients ($n=13$ male) in whom only lumbar spine was reported due to absence of age match control for femoral neck. Of the 27 patients found to have low baseline BMD's, $n=15$ (75%) males and $n=3$ (42.8%) females were treated with combination of bisphosphonate and calcium/vit D supplements. $n=1$ female and $n=1$ male received calcium/vit D only. eight ($n=4$ female) were untreated.

Fourteen patients with low baseline BMD's ($n=3$ female) had follow-up scans. In the group treated with combination of bisphosphonate and calcium/vit D supplement, nine ($n=$eight males) demonstrated improved BMD when rescanned. $n=2$ males and $n=1$ female showed no improvement and $n=2$ (one male) showed reduction in BMD. Three male patients had normal baseline BMD's and there was no change in the intervening 2–4 years.

Conclusion

Two-thirds of the patients attending transitional clinic were found to have low BMD's. Baseline scans would suggest that females in this age group have a higher risk of developing low BMD. In those patients who received combination of bisphosphonate and calcium/vit D; 64% males and 33% females showed improvement in BMD when rescanned.

DOI: 10.1530/endoabs.34.P12

P13

Mutational analysis of the adaptor protein 2 sigma subunit *(AP2S1)* gene: search for autosomal dominant hypocalcaemia type 3 (ADH3)

Angela Rogers[1], M Andrew Nesbit[1], Fadil M Hannan[1], Sarah A Howles[1], Treena Cranston[2], Jeremy Allgrove[3,4], John S Bevan[5], Gul Bano[6], Caroline Brain[3], Vipan Datta[7], Shirley V Hodgson[8], Louise Izatt[9], Lynne Millar-Jones[10], Simon H Pearce[11], Lisa Robertson[12], Peter L Selby[13], Brian Shine[14], Katie Snape[8], Justin Warner[15] & Rajesh V Thakker[1]
[1]Academic Endocrine Unit, Radcliffe Department of Medicine, University of Oxford, Oxford, UK; [2]Oxford Molecular Genetics Laboratory, Churchill Hospital, Oxford, UK; [3]Department of Paediatric Endocrinology, Great Ormond Street Hospital, London, UK; [4]Department of Paediatric Endocrinology, Royal London Hospital, London, UK; [5]Department of Endocrinology, Aberdeen Royal Infirmary, Aberdeen, UK; [6]Department of Diabetes and Endocrinology, St George's Hospital, London, UK; [7]Jenny Lind Children's Department, Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, UK; [8]Department of Clinical Genetics, St George's Hospital, University of London, London, UK; [9]Department of Clinical Genetics, Guy's and St Thomas' Foundation Trust, Guy's Hospital, London, UK; [10]Department of Paediatrics, Royal Glamorgan Hospital, Glamorgan, UK; [11]Institute of Genetic Medicine, Newcastle University, Newcastle Upon Tyne, UK; [12]Department of Clinical Genetics, Leicester Royal Infirmary, Leicester, UK; [13]Department of Medicine, Manchester Royal Infirmary, Manchester, UK; [14]Department of Clinical Biochemistry, John Radcliffe Hospital, Oxford, UK; [15]Department of Paediatrics, University Hospital of Wales, Cardiff, UK.

Familial hypocalciuric hypercalcaemia types 1, 2, and 3 (FHH1, FHH2, and FHH3) are caused by loss-of-function mutations of the calcium-sensing receptor (CaSR), G-protein subunit α11 (Gα11) and adaptor protein 2 sigma subunit (AP2σ), respectively; whilst autosomal dominant hypocalcaemia types 1 and 2 (ADH1 and ADH2) are due to gain-of-function mutations of CaSR and Gα11, respectively. We therefore hypothesised that gain-of-function AP2σ mutations may result in ADH3, and investigated ADH patients who did not have CaSR or Gα11 mutations, for DNA sequence abnormalities of the *AP2S1* gene, which encodes AP2σ. Sixteen patients (12 males and four females) with hypocalcaemia (albumin-adjusted calcium levels ranging from 0.94–2.03 mmol/l, normal range 2.10–2.60 mmol/l) in association with low or normal parathyroid hormone concentrations (ranging from <0.7–5.6 pmol/l, normal range 1.3–7.6 pmol/l; and from 2–26 ng/l, normal range 10–65 ng/l), consistent with ADH, but who did not have CaSR or Gα11 mutations, were included in the study. The 16 hypocalcaemic patients ranged in age at diagnosis or presentation from the neonatal period to 68 years old and 50% of patients presented with seizures or hypocalcaemic symptoms. More than 60% of the patients ($n=16$) had elevated serum phosphate concentrations. The urinary calcium:creatinine clearance ratio was high in 30% of thirteen patients. Leukocyte DNA was used for sequence analysis of the entire coding region and exon–intron boundaries of AP2S1.
However, *AP2S1* coding region mutations were not detected in these 16 hypocalcaemic patients. Binomial probability analysis, using the assumption that *AP2S1* mutations would occur in hypocalcaemic patients, at a prevalence of 20%, similar to that observed in FHH patients without CaSR or Gα11 mutations, indicated that the likelihood of detecting at least one *AP2S1* mutation was >97% in this sample size of 16 hypocalcaemic patients. Thus, our findings suggest that ADH3 due to gain-of-function AP2σ mutations may be rare or non-existent.

DOI: 10.1530/endoabs.34.P13

P14

Clinical studies of adaptor protein-2 sigma subunit mutations causing familial hypocalciuric hypercalcaemia type 3 reveal genotype–phenotype correlations and effectiveness of cinacalcet

Fadil Hannan[1], Angela Rogers[1], Sarah Howles[1], Treena Cranston[2], Malachi McKenna[3], Tristan Richardson[4], Valerie Babinsky[1], Anita Reed[1], Clare Thakker[1], Detlef Bockenhauer[5], Rosalind Brown[6], Jacqueline Cook[7], Ken Darzy[8], Sarah Ehtisham[9], Una Graham[10], Tony Hulse[11], Steven Hunter[10], Dhavendra Kumar[12], John McKnight[13], Patrick Morrison[14], Zulf Mughal[9], Simon Pearce[15], Isabelle Scheers[16], Timothy Wang[17], Michael Whyte[18], M Andrew Nesbit[1] & Rajesh Thakker[1]
[1]University of Oxford, Oxford, UK; [2]Churchill Hospital, Oxford, UK; [3]St. Vincent's University Hospital, Dublin, Ireland; [4]Royal Bournemouth Hospital, Bournemouth, UK; [5]UCL Institute of Child Health and Great Ormond Street Hospital for Children, London, UK; [6]Boston Children's Hospital, Boston, Massachusetts, USA; [7]Sheffield Children's Hospital, Sheffield, UK; [8]Queen Elizabeth II Hospital, Welwyn Garden City, UK; [9]Royal Manchester Children's Hospital, Manchester, UK; [10]Royal Victoria Hospital, Belfast, UK; [11]St Thomas' Hospital, London, UK; [12]University Hospital of Wales, Cardiff, UK; [13]Western General Hospital, Edinburgh, UK; [14]Queen's University Belfast, Belfast, UK; [15]Newcastle University, Newcastle Upon Tyne, UK; [16]Cliniques universitaires Saint-Luc, Brussels, Belgium; [17]Frimley Park Hospital, Surrey, UK; [18]Shriners Hospital for Children, St. Louis, Missouri, USA.

Familial hypocalciuric hypercalcaemia (FHH) comprises three types: FHH1, FHH2, and FHH3, which are due to mutations of the calcium-sensing receptor (CaSR), G-protein α 11 subunit (Gα11), and adaptor protein-2 sigma subunit (AP2σ), respectively. The aims of this study were: to assess for genotype–phenotype correlations among the three reported FHH3-causing AP2σ mutations, which all involve the Arg15 residue, and comprise Arg15Cys, Arg15His, and Arg15Leu; to compare the calcium-related phenotypes between FHH3 and FHH1; and to assess the effectiveness of cinacalcet, a CaSR-targeted agonist, to lower the hypercalcaemia in FHH3 individuals. An analysis of genotype–phenotype correlations in the FHH3 probands ($n=23$) harbouring either the Arg15Cys ($n=7$), Arg15His ($n=7$) or Arg15Leu ($n=9$) AP2σ mutations, revealed Arg15Leu to be associated with marked hypercalcaemia (mean ± S.E.M serum adjusted-calcium = 3.08 ± 0.05 mmol/l for Arg15Leu, 2.83 ± 0.04 mmol/l for Arg15Cys, and 2.79 ± 0.05 mmol/l for Arg15His, $P<0.01$). A comparison of the biochemical phenotypes of FHH1 individuals ($n=43$) and FHH3 individuals ($n=48$), revealed FHH3 to be associated with significantly higher serum adjusted-calcium concentrations (2.87 ± 0.02 mmol/l for FHH3 vs 2.76 ± 0.02 mmol/l for FHH1, $P=0.001$) and significantly lower urinary calcium-to-creatinine clearance ratios (0.004 ± 0.001 for FHH3 vs 0.007 ± 0.001 for FHH1, $P<0.001$). There were no significant differences in serum concentrations of phosphate, alkaline phosphatase activity, or parathyroid hormone (PTH) between FHH3 and FHH1 subjects. Administration of cinacalcet (30–60 mg daily for >6 months) in three unrelated symptomatic FHH3 individuals, each with a Arg15Cys, Arg15His, or Arg15Leu mutation and with serum calcium concentrations ≥3.0 mmol/l, corrected the values to within the normal range, and also lowered serum PTH concentrations, which remained within the normal range. These findings indicate a genotype–phenotype correlation in FHH3 individuals, with the Arg15Leu AP2σ mutation resulting in the most severe FHH phenotype, and demonstrate the effectiveness of cinacalcet in correcting the hypercalcaemia of FHH3 individuals.

DOI: 10.1530/endoabs.34.P14

P15

Identification of 12 adaptor protein-2 sigma 2 subunit mutations in familial hypocalciuric hypercalcaemia type 3 and expansion of phenotypic spectrum.

Angela Rogers[1], Fadil M Hannan[1], Sarah A Howles[1], Treena Cranston[2], Clare E Thakker[1], Detlef Bockenhauer[3], Rosalind Brown[4], Jacqueline A Cook[5], Ken Darzy[6], Sarah Ehtisham[7], Tony Hulse[8], Dhavendra Kumar[9], Malachi McKenna[10], John McKnight[11], Zulf Mughal[7], Simon H Pearce[12], Tristan Richardson[13], Isabelle Scheers[14], Timothy Wang[15], M Andrew Nesbit[1] & Rajesh V Thakker[1]
[1]Academic Endocrine Unit, Radcliffe Department of Medicine, University of Oxford, Oxford, UK; [2]Oxford Molecular Genetics Laboratory, Churchill Hospital, Oxford, UK; [3]UCL Institute of Child Health and Great Ormond Street Hospital for Children, London, UK; [4]Division of Endocrinology, Boston Children's Hospital, Boston, Massachusetts, USA; [5]Clinical Genetics Department, Sheffield Children's Hospital NHS Foundation Trust, Sheffield, UK; [6]Queen Elizabeth II Hospital, Welwyn Garden City, UK; [7]Department of Paediatric Endocrinology, Royal Manchester Children's Hospital, Manchester, UK; [8]Department of Paediatrics, St Thomas' Hospital, London, UK; [9]Department of Clinical Genetics, University Hospital Wales, Cardiff, UK; [10]Department of Endocrinology, St Vincent's University Hospital, Dublin, Ireland; [11]Department of Endocrinology and Diabetes, Western General Hospital, Edinburgh, UK; [12]Institute of Genetic Medicine, Newcastle University, Newcastle Upon Tyne, UK; [13]Department of Endocrinology, Royal Bournemouth Hospital, Bournemouth, UK; [14]Department of Paediatrics, Cliniques Universitaires Saint-Luc, Brussels, Belgium; [15]Clinical Biochemistry Department, Frimley Park Hospital, Surrey, UK.

Familial hypocalciuric hypercalcaemia (FHH) is an autosomal dominant disorder characterized by hypercalcaemia and inappropriately low urinary calcium excretion, and is occasionally associated with acute pancreatitis. FHH can be classified into three types: FHH type 1, caused by loss-of-function mutations of the calcium-sensing receptor (CaSR), which accounts for >65% of cases; FHH type 2,

due to loss-of-function mutations of the G-protein α 11 subunit (Gα11), of which two cases have been identified; and FHH type 3 resulting from loss-of-function mutations in the ubiquitously expressed adaptor protein-2 sigma subunit (AP2σ), encoded by AP2S1, of which 14 patients and families have been reported. The aim of this study was to investigate a further 45 FHH probands (15 males and 30 females), who did not harbour CaSR or Gα11 mutations, for the presence of AP2σ mutations. Leukocyte DNA was used for mutational analysis of the entire coding region and exon–intron boundaries. DNA sequence analysis identified AP2σ mutations in 12 probands (Four males and eight females, age at diagnosis ranging from 9 years to 48 years old), which all affected the Arg15 residue, and comprised three Arg15Cys, five Arg15Leu, and four Arg15His AP2σ mutations. Two unrelated, female patients aged 14 and 15 years old harboured *de novo* Arg15Leu AP2σ mutations; the 15-year-old girl also had short stature, learning difficulties and an atrial-septal defect, and the 14-year-old girl also had short stature, learning difficulties and recurrent episodes of acute pancreatitis. In summary, 12 AP2σ mutations were identified in 45 FHH probands, indicating that AP2σ mutations account for >25% of FHH patients without CaSR and Gα11 mutations. Thus, our findings indicate that *AP2S1* mutational analysis should be undertaken in FHH patients who do not have CaSR mutations, and that patients harbouring an Arg15Leu AP2σ mutation may present with additional non-calciotropic phenotypes.

DOI: 10.1530/endoabs.34.P15

P16

Role of cortisol in the pathogenesis of postmenopausal osteoporosis: relationship to bone structure

Miguel Debono[1], Selina Bratherton[1], Margaret Paggiosi[1], Fatima Gossiel[1], Brian Keevil[2], Richard Ross[1] & Richard Eastell[1]
[1]University of Sheffield, Sheffield, South Yorkshire, UK; [2]University Hospital of South Manchester, Manchester, North West, UK.

Background
Excess glucocorticoids are well recognised as a cause of osteoporosis; they inhibit osteoblast function and increase osteoblast and osteocyte apoptosis resulting in thinning of the trabeculae. The circadian rhythm of bone turnover, which is linked to cortisol rhythm, is abnormal in osteoporosis. Furthermore, some studies show abnormal cortisol metabolism in osteoporosis. The aim of our study was to evaluate the day–night rhythm of cortisol and to relate cortisol levels to bone micro-structure.
Methods
Postmenopausal women with vertebral fractures and a hip or spine BMD *T*-score < -1.0 were recruited (Group 1, $n=30$) In addition, two sex-/age-matched control groups with i) BMD matched to those in Group 1 (Group 2, $n=30$), and ii) normal BMD (Group 3, $n=30$) were studied. Salivary cortisol was measured at 0900 and 2300 h. Lumbar spine and hip BMD were measured by DXA, prevalent vertebral fractures were identified by VFA, and bone micro-structure in the distal radius and tibia was examined by high resolution peripheral quantitative computed tomography (HR-pQCT). Vertebral fractures were confirmed on plain radiography using the algorithm-based qualitative method.
Results
Between group differences in hip ($P<0.001$) and spine ($P<0.001$) *T*-scores were detected by ANOVA. *Post-hoc* Scheffe tests revealed that spine and hip *T*-scores for Groups 1 and 2 did not differ, whilst *T*-scores for Group 3 were higher ($P<0.001$). Mean (s.d.) 0900 h salivary cortisol was significantly elevated in the vertebral fracture group (9.2 (4.7), 6.6 (5.0)and 6.4 (4.1) nmol/l ANOVA by trend $P=0.034$) and this correlated significantly with radial total volumetric BMD (Spearman's $\rho -0.78$; $P=0.01$) and trabecular thickness (Spearman's $\rho -0.73$; $P=0.026$) in the vertebral fracture group. 2300 h salivary cortisol levels were not related to BMD or bone micro-structure.
Conclusion
Our findings show that the diurnal cortisol rhythm may possibly be altered in those women with vertebral fractures. Higher morning cortisol could contribute to an increased risk of vertebral fractures through thinning of the trabeculae.

DOI: 10.1530/endoabs.34.P16

P17

The calcilytic NPS2143 rectifies the gain-of-function associated with G-protein α 11 mutations causing autosomal dominant hypocalcaemia type 2

Valerie Babinsky[1], Fadil Hannan[1], M Andrew Nesbit[1], Sarah Howles[1], Jianxin Hu[2], Allen Spiegel[1] & Rajesh Thakker[1]
[1]University of Oxford, Oxford, UK; [2]National Institutes of Health, Bethesda, Maryland, USA; [3]Albert Einstein College of Medicine, Bronx, New York, USA.

Autosomal dominant hypocalcaemia (ADH) is a disorder that needs to be distinguished from hypoparathyroidism, as ADH patients are at risk of nephrocalcinosis and renal failure when treated with activated vitamin D preparations. ADH types 1 and 2 are due to gain-of-function mutations of the calcium-sensing receptor (CaSR) and G-protein α 11 (Gα11), respectively. CaSR targeted drugs, known as calcilytics, rectify the gain-of-function associated with ADH1-causing mutations. However, it is unclear whether calcilytics have potential for the treatment of ADH2. The aim of this study was to determine whether a calcilytic agent, known as NPS2143, may ameliorate the gain-of-function associated with ADH2-causing Gα11 mutations. WT and gain-of-function Gα11 mutants (Arg181Gln and Phe341Leu) were expressed by transient transfection in HEK293 cells stably transfected with CaSR and assessed using a quantitative immunoassay (AlphaScreen) to measure levels of phosphorylation of a downstream signaling protein, known as extracellular signal regulated kinase (ERK), in responses to changes in extracellular calcium concentrations ($[Ca^{2+}]_o$) in the presence and absence of NPS2143. These studies demonstrated that both Arg181Gln and Phe341Leu Gα11 mutations led to a leftward shift of the concentration–response curves and significantly ($P<0.0001$) reduced mean half-maximal (EC_{50}) values (Arg181Gln $EC_{50}=2.29$ mM, 95% (CI)=2.25–2.34; Phe341Leu $EC_{50}=2.30$ mM, 95% CI=2.25–2.35) when compared to WT ($EC_{50}=2.80$ mM, 95% CI=2.74–2.86). The addition of 20 nM of the calcilytic NPS1243 led to significant ($P<0.0001$) rightward shifts of the concentration–response curves and increase in the EC_{50} for Arg181Gln ($EC_{50}=3.96$ mM, 95% CI=3.90–4.02) and Phe341Leu ($EC_{50}=3.38$ mM, 95% CI=3.33–3.43) when compared to untreated Gα11 mutants. Thus, our findings demonstrate that NPS2143 corrects the gain-of-function associated with ADH2-causing Gα11 mutations, and indicates that calcilytic drugs have potential for the treatment of this hypocalcaemic disorder.

DOI: 10.1530/endoabs.34.P17

P18

Baseline characteristics of patients presenting with primary hyperparathyroidism

Alice Blackwell, Zana Ameen, Sarah Goellner, Abd Tahrani, Andrew Bates & Asad Rahim
Heart of England NHS Foundation Trust, Birmingham, UK.

Introduction
Guidelines have, for some time, suggested a lower threshold for surgical intervention in primary hyperparathyroidism based on serum calcium alone. Furthermore, PHPT is a common metabolic bone disorder which is associated with further complications. In order to assess the impact of the lower threshold on surgical services and management implications for associated complications, we report baseline characteristics in a cohort of patients with confirmed PHPT.
Method
Retrospective analysis of 125 patients to collect baseline data for corrected calcium ($n=125$), vitamin D ($n=119$), PTH ($n=125$), bone mineral density (BMD) of the lumbar spine ($n=71$) and neck of femur (NOF) ($n=72$), renal imaging (CT or US scan; $n=83$). Finally, we report the number of parathyroid US scans ($n=62$) and MIBI scans ($n=57$) demonstrating concordance.
Results
Mean (±s.d.) age was 64.8 (±16.5) years. Mean (±s.d.) corrected calcium, PTH, and vitamin D at baseline were 2.7 mmol/l (±0.21), 18.6 pmol/l (±10.2), and 32.3 nmol/l (±23.5) respectively.
The number of patients with serum calcium between 2.85 and 2.99 and >3.0 mmol/l was 14 and 20 respectively. Of those with vitamin D deficiency, 74 (60%) were deficient (<30 mmol/l), 25 (20%) were insufficient (30–50 mmol/l). Of those scanned, DEXA demonstrated reduced BMD at the lumbar spine with 28 (39%) osteopenic and 14 (20%) osteoporotic. For NOF, 20 (28%) were osteopenic and 11 (15%) osteoporotic. Of 83 (66%) who had renal imaging, 18 (21.7%) demonstrated renal tract calcification.
62 (50%) underwent parathyroid ultrasound, 19 (31%) had an identifiable adenoma.
57 (46%) underwent a MIBI scan, 20 (35%) showed an adenoma.
10/57 (17.5%) patients had an adenoma on both.
Conclusion
Based on current guidelines, the number of referrals for surgical treatment on calcium levels alone has increased by 70%. Furthermore, assessment and treatment of complications prior to surgery, i.e. vitamin D deficiency, must be considered as well as follow-up of BMD/renal complications.

DOI: 10.1530/endoabs.34.P18

P19

Treatment of vitamin D deficiency in primary hyperparathyroidism (PHPT) with different vitamin D preparations.

Sarah Goellner, Zana Ameen, Alice Blackwell, Abd Tahrani, Andrew Bates & Asad Rahim
Heart of England NHS Foundation Trust, Birmingham, UK.

Introduction
Eighty percent of patients with PHPT have co-existing vitamin D deficiency. Few large studies have assessed the impact/safety of different vitamin D preparations on calcium, PTH and vitamin D in such patients. We report the use of three different preparations.
Methods
In a retrospective study of 125 patients with confirmed PHPT, 77% were vitamin D deficient (<30 nmol/l)/insufficient (30–50 nmol/l). We assessed the impact and safety of treatment with vitamin D3 or D2 (50 000 IU) after 48 weeks ($n=35$), calcium and vitamin D (e.g. Adcal-D3) twice daily for 3 months ($n=16$) and over the counter (OTC) vitamin D 1000 IU/day ($n=41$) for 3 months on calcium, PTH and vitamin D levels. In four patients, the Vitamin D preparations were unspecified.
Results
See Table. Mean (±S.D.) serum calcium remained stable after treatment for whole group (2.75 (±0.22) vs 2.74 (±0.24) mmol/l; $P=0.699$).
Conclusion
Vitamin D repletion, regardless of regimen, in patients with PHPT and Co-existing vitamin D deficiency is safe and does not significantly exacerbate hypercalcaemia for the majority of patients. Patients treated with combined calcium and vitamin D preparations had lower baseline calcium, but calcium levels still did not rise significantly with treatment.
Finally, despite the increased metabolism of vitamin D in PHPT, most patients were replete within 3–6 months regardless of vitamin D preparation.

Table 1

Vit D prep	Corrected calcium (mmol/l)			Vitamin D (nmol/l)			PTH (pmol/l)		
	Pre-treat mean (±S.D.)	Post-treat mean (±S.D.)	P value	Pre-treat mean (±S.D.)	Post-treat mean (±S.D.)	P value	Pre-treat mean (±S.D.)	Post-treat Mean (±S.D.)	P value
50000IU	2.78 (±0.23)	2.76 (±0.24)	0.50	26.8 (±20.4)	72.5 (±35.5)	<0.01	21.2 (±11.9)	17.2 (±8.4)	0.02
1000IU	2.75 (±0.24)	2.76 (±0.27)	0.89	29.2 (±15.0)	70.0 (±28.5)	<0.01	20.3 (±11.0)	21.8 (±27.2)	0.73
Ca/Vit D	2.63 (±0.13)	2.65 (±0.20)	0.81	32.6 (±27.4)	63.5 (±30.6)	<0.01	17.5 (±8.2)	12.0 (±6.3)	0.01

DOI: 10.1530/endoabs.34.P19

P20

Is parathyroid hormone venous sampling useful? Correlation of parathyroid hormone selective venous sampling and histopathological results in patients who underwent parathyroidectomy between 2006 and 2013

Andrzej Rys, Robert Dyer, David Cunliffe, Simon Hickey & Richard Seymour
Department of Endocrinology and Diabetes, South Devon Healthcare NHS Foundation Trust, Torbay Hospital, Torquay, UK.

Patients with primary hyperparathyroidism (PHPT) undergo parathyroid ultrasound, Tc-99 m sestamibi scan or MRI to localize hyperfunctioning parathyroid gland(s). When scans are negative or discordant we perform parathyroid hormone selective venous sampling (PTHSVS).
We report the results of 17 patients (Four males, 13 females, average age 58.6) with PHPT, who underwent PTHSVS followed by either focused parathyroidectomy (FP) or bilateral neck exploration (BNE) in years 2006–2013.
All patients had both parathyroid ultrasound and Tc-99 m sestamibi scans. Parathyroid ultrasound showed lateralisation in 26.3% (15.8% possible and 10.5% confident) and Tc-99 m sestamibi showed lateralisation in 36.8% (31.6% possible and 5.3% confident). In 11.8% of patients lateralisation was present on both scans but results were discordant.
On average during the PTHSVS procedure samples for PTH levels were obtained from 10.67 sites. All procedures were successful.
PTHSVS showed lateralisation in 11 patients (64.1%). Following the results of PTHSVS nine patients underwent FP and two had BNE. Histopathological results confirmed 9 adenomas (81.8% true positive). In remaining two cases (18.2% false positive) there was one confirmed hyperplasia and one positive PTHSVS is thought likely to contain a spurious PTH result.
The average ratio between the site of highest PTH level and level of PTH in inferior vena cava was 7.68 (2.61–19.38) in patients with lateralisation.
In six patients with no lateralisation on PTHSVS, three had BNE and 3 FP. Histopathological report in five patients confirmed existence of parathyroid adenoma (83.3% false negative). One patient had hyperplasia (16.7% true negative).
Seven out of nine patients who had FP were cured during first operation. Two patients required reoperation, one of them was not cured due to anatomical localization of the adenoma.
Our report suggests that PTHSVS is a helpful diagnostic adjunct in localization of hyperfunctioning parathyroid gland(s). Positive PTHSVS increases the surgeon's confidence in choosing a less invasive procedure.

DOI: 10.1530/endoabs.34.P20

P21

Predictors of postoperative hypocalcaemia in parathyroidectomy patients: local audit

Venkata Katreddy, David Blundell & Harit Buch
Wolvorhampton Diabetes Centre, Wolverhampton, UK.

Introduction
Parathyroidectomy is the treatment of choice for patients with primary hyperparathyroidism who have significant hypercalcemia and/or end-organ damage. The incidence of postoperative hypocalcaemia is 25–90% in patients with evidence of radiological evidence of parathyroid disease, 0–6% without. In most patients it is mild, but in some, it can cause significant morbidity. It has been attributed as 'hungry-bone syndrome' and attempts have been made to identify predictors with a view to reducing its incidence.
Methods
We retrospectively reviewed patients who had parathyroidectomy over the past 3 years at our hospital and assessed the frequency of post-operative hypocalcaemia (serum calcium <2.1 mmol/l). We correlated the occurrence of hypocalcaemia with predictors known to influence the frequency of its occurrence.
Results
46 patients had surgery over 3-year period. Mean age 63 years, 12 (26%) were males. Mean preoperative calcium, parathyroid hormone, and alkaline phosphatase were 2.95 mmol/l (2.1–2.6), 20.53 pmol/l (1.6–7.2), and 102 IU/l (30–130) respectively. Pre-operative vitamin D level was recorded in 22 patients and 10/22 had significant vitamin D deficiency (<25 nmol/l). Mean weight of parathyroid adenoma was 2.5 g. Nine patients (20%) had post-operative hypocalcaemia with mean calcium of 1.92 mmol/l (+0.33). All patients had an eventless course and spontaneous recovery. There was no statistically significant difference between those who developed hypocalcaemia and those did not, in terms of mean age, gender distribution, pre-operative serum calcium, parathyroid hormone and alkaline phosphatase or with the weight of parathyroid adenoma ($P>0.05$ for all). Unfortunately as pre-operative vitamin D level was only available in 50% of patients; its correlation with hypocalcaemia could not be ascertained reliably.
Conclusion
The frequency of post parathyroidectomy hypocalcaemia was high in our patients. This may be related to high prevalence of vitamin D deficiency. However we did not find correlation between the occurrence of post-operative hypocalcaemia and any of its known predictors.

DOI: 10.1530/endoabs.34.P21

P22

A rare germline Leu63Pro missense mutation in *CDC73* resulting in familial primary hyperparathyroidism with variable phenotype

Khyatisha Seejore, Robert Murray, Stephen Orme & Afroze Abbas
Department of Endocrinology, St James's University Hospital, Leeds, UK.

Introduction
Primary hyperparathyroidism (PHPT) is a common endocrine disorder. However, a familial hyperparathyroid syndrome is diagnosed in less than 5% of cases. We present two related cases of *CDC73*-related familial hyperparathyroidism due to a rarely described germline Leu63Pro missense mutation in *CDC73* exon 2.
Case report
The index patient, a 24-year-old female, presented acutely unwell with symptoms of hypercalcaemia. Her blood tests showed PTH 186.1 pmol/l (1.5–7.6) with calcium 4.20 mmol/l (2.2–2.6). Parathyroid imaging demonstrated bilateral parathyroid adenomas. Her life-threatening hypercalcaemia was treated with i.v. saline and bisphosphonates. However despite treatment with cinacalcet, persistent severe symptomatic hypercalcaemia necessitated an urgent parathyroidectomy. All four parathyroid glands were excised. Her recovery was complicated by severe hungry bone syndrome.
The patient's parents are unaffected by hyperparathyroidism. However it transpired that her 35-year-old brother had a history of resected Wilms' tumour aged 5. He also had PHPT with a left inferior parathyroid adenoma removed in 2008. Despite being initially lost to follow-up, he was subsequently re-referred with symptomatic recurrent PHPT (PTH 31.2 pmol/l, Ca 2.95 mmol/l). He underwent repeat parathyroid surgery and one normally functioning parathyroid gland was left in-situ. In both cases, parathyroid histology was benign. Genetic testing revealed germline Leu63Pro missense mutation in *CDC73* exon 2.
Discussion
CDC73-related familial hyperparathyroidism encompasses a spectrum of parathyroid disorders including hyperparathyroidism-jaw tumour syndrome (HPT-JT). HPT-JT is an autosomal dominant condition with incomplete penetrance, characterised by atypical parathyroid tumours ($\sim$15% malignant) with tumours of one or more of the jaw, kidneys or uterus. Patients are at high risk of recurrent parathyroid and non-parathyroid tumours and require long-term surveillance. These particular cases demonstrate an unusual germline missense mutation of CDC73 in the same family but with variable phenotype.

DOI: 10.1530/endoabs.34.P22

P23

Hypogonadism masquerading as metabolic bone disease in an young male

Mallikarjuna Jeeragi, Arpandev Bhattacharyya, S Vidhyadhara & Karthik Prabhakar
Manipal Hospitals, Bangalore, Karnataka, India.

A 38-year-old farmer presented to Orthopaedics Department with backache and progressively increasing difficulty in walking for a year. MRI spine showed multiple central vertebral fractures suggestive of possible metabolic bone disease/oncogenic osteomalacia; he was hence referred to Endocrinology Department.
He was in pain. No previous history of trauma, no gastrointestinal or urinary symptoms. He however reported erectile dysfunction. On examination he was not Cushingoid, had generalized spinal tenderness and small testicles, but secondary sexual characteristics were normal. Initial evaluation showed normal FBC, U&Es but ESR 71(0–10 mm/h) and CRP 67 mg/l ($<$6) were high. DEXA scan revealed osteoporosis: *T*-scores at lumbar spine $-$4.1, Neck-of-Femur $-$4.0 and Forearm $-$4.0. Serum corrected calcium 9.4 mg/dl (8.8–10.6), magnesium 1.8 mg/dl (1.6–2.6), PTH 20.6 pg/ml (10–65), alkaline phosphatase 124 U/l (53–128) were normal; vitamin-D 18.5ng/ml ($>$30) low; urine pH 5, serum bicarbonate 24.6 mmol/l (21–31) both normal; dexamethasone-suppression test excluded cortisol-excess. 9am testosterone 30 pg/ml (50–210) was low but FSH 5.6 mIU/ml (2–10) and LH 5.1 mIU/ml (1.4–11) were inappropriately normal; prolactin 10 ng/ml (3–18), TSH 4.7 μIU/ml (0.3–5–5) and freeT_4 1.7 ng/dl (0.3–2.3), PSA were within-limits. Pituitary Imaging was normal. Urine/ serum immunofixation were negative for light-chains, serum immunofixation was negative for monoclonal-gammopathy, urinary Bence-Jones proteins were negative and bone-marrow biopsy was negative for hematolymphoid malignancy. PET-CT done showed no neoplastic/metastatic lesions. Bone scan too suggested metabolic bone disease.
Having excluded underlying malignancy and multiple myeloma by various investigations, it was concluded that significant osteoporosis (metabolic bone disease) is possibly due to hypogonadism plus vitamin D deficiency. He was treated with calcium + vitamin D supplements, Androgen-replacement-therapy and physiotherapy. By 3 months, he made good clinical recovery, and radiological evidence of healed vertebral fractures.
Osteoporosis, characterized by decreased bone-mineral-density and increased fracture-risk, is common among females and the elderly. This case illustrates the difficulties in evaluation and management of young male patient with severe osteoporosis.

DOI: 10.1530/endoabs.34.P23

P24

Unusual parathyroid location: a case of primary hyperparathyroidism with failed right parathyroidectomy

Tannaz Vakilgilani, Youngyoung Ling, David Woods, Agnieszka Falinska & Jeannie F Todd
Imperial Centre of Endocrinology, Hmmersmith Hospital, London, UK.

19-year-old gentleman was initially investigated by his local hospital when he presented with 2-year history of diarrhoea, intermittent abdominal pain, sweating, and depression with suicidal ideation. His only past medical history was eczema. His maternal grandmother had PTHP aged 50 years. A paternal grandmother had colon and pancreatic cancer and cousin had Graves' disease.
Gastroenterology investigations were normal. However he was found to have hypercalcaemia with elevated, PTH and high 24 h urine calcium:creatinine ratio which was consistent with PHPT. USS was suggestive of right lower parathyroid adenoma but sestamibi scan was inconclusive. He had a right inferior prathyroidectomy but histology showed normal parathyroid and he remained hypercalcaemic post-operatively.
He was then referred to Hammersmith hospital for further investigation. His symptoms continued post-operatively. Repeat investigations confirmed PTHP with a 24 h calcium:creatinine ratio of 0.017, calcium: 2.82, PO_4: 1.17 and an elevated PTH. He had no nephrocalcinosis in USS but osteopenia in DEXA scan. In addition, in view of his young age it is our routine practice to exclude MEN 1. A biochemical screen and genetic test for MEN 1 was performed and it was negative for a mutation. His pituitary function and gut hormones profile were normal.
He had further localisation studies were unhelpful with negative repeat sestamibi scan USS and CT scan of the neck. Therefore he underwent PTH sampling which showed a 10 $\times$ 5 mm diameter oval vascular blush on the left side of the neck with concordant rise in PTH levels at this site. Although CT scan had been reported as normal, in retrospect this did revealed 10 $\times$ 5 mm diameter oval soft tissue abnormality lying between left side of the trachea and the left lobe of the thyroid. He is now awaiting surgical removal of the parathyroid adenoma.
This case highlights that PTH adenomas may be found in unusual places particularly if the localization studies are negative. We would also recommend MEN 1 genetic screen in any patient who develops PHPT below the age 40 years.

DOI: 10.1530/endoabs.34.P24

P25

Is the response to high dose oral vitamin D replacement predictable?

Deepak Chandrajay, Marina Milic, Gwen Al-Khaili, Afroze Abbas & Stephen Orme
St James University Hospital, Leeds, UK.

In our institution, an observed loading dose of oral colecalciferol (D3) 300 000 units is used for treatment of vitamin D deficiency (Vit D $<$30 nmol/l), with subsequent re-evaluation at 6 weeks and 3 months. We evaluated the follow-up of all the patients who received loading dose colecalciferol against adherence to this protocol.
Method
All patients who received observed loading dose of colecalciferol for a 1-year period were included. Demographic, clinical, and biochemical data was collected from existing databases and clinical notes.
Results
A total of 256 patients had observed loading dose colecalciferol (female 179 and male 77). The age range was 18–91 years old. The median age was 54.62 years. Basal Vit D at time of loading ranged between $<$20 and 43.5 nmol/l.

Vit D was repeated at 6 weeks and 3 months, as per protocol in only 63 and 48% of cases respectively. Of those who did have Vit D rechecked at 6 weeks, 87% ($n=140/161$) improved their Vit D concentration >50 nmol/L, 9.3% ($n=15$) had levels between 20–50 nmol/l and only 3.7% ($n=6$) had Vit D <20 nmol/l. Vitamin D concentrations fell to <50 nmol/l in 48% (74/154) of the patients within 12 months.
18.75% ($n=48$) patients required oral reloading within 12 months and seven were switched to i.m. ergocalciferol. Four of these patients were known to have malabsorption problems. There was no correlation between BMI and the response to high dose Vit D or need for further oral reloading. There were no episodes of toxicity or adverse events. There was no significant hypercalcaemia.
Conclusion
Observed loading dose colecalciferol was safe and efficacious in this heterogeneous group of patients. Problems with adherence to the protocol were identified which led to need for reloading in a large group of patients. BMI had no impact on response to high dose Vit D.

DOI: 10.1530/endoabs.34.P25

P26

Functional read out of defective osteoclast function in an *in vitro* model of Gaucher's disease

Sarbjit Nijjar[1], Neil Gittoes[2] & Tarekegn Geberhiwot[2]
[1]School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, Centre for Endocrinology, Diabetes, and Metabolism, University of Birmingham, Edgbaston, Birmingham B15 2TT, West Midlands, UK; [2]Department of Medicine Old QEH and 2nd Floor Wolfson Endocrinology, University Hospitals Birmingham NHS Foundation Trust, Queen Elizabeth Medical Centre, Queen Elizabeth Hospital, Birmingham B15 2TH, West Midlands, UK.

Gaucher's disease is an inherited disorder caused by loss or reduced activity of the lysosomal enzyme glucocerebrosidase (GBA). Nearly 80% of patients with Gaucher's disease develop abnormal bone remodelling with severe consequences, including bone crises, osteonecrosis and osteoporosis related fractures. Although enzyme replacement therapy is effective at alleviating most manifestations of the disease, only modest improvements in bone health can be achieved. The reason for this apparent 'bone resistance' is unclear but may reflect abnormal osteoclast function. To address this problem, we developed an *in vitro* model of osteoclast bone resorption, in which we differentiated the mouse cell line RAW264.7, into functional osteoclasts using RANKL. Multinucleated tartrate resistant alkaline phosphatase (TRAP) positive cells were apparent after 7 days of culture on uncoated tissue culture plastic.
Osteoclasts were differentiated on inorganic calcium phosphate coated plates designed to mimic the *in vivo* bone environment. Cultures were maintained for 14 days and the Gaucher's defect was modelled by culturing cells in the presence of conduritol-β-epoxide (CBE), a potent, irreversible inhibitor of mammalian GBA. After the culture period, cells were removed and the number of resorption pits counted. We observed that the number of osteoclasts and resorption pits was increased by twenty percent following culture in the presence of 50 μM CBE and this effectively doubled at 100 μM.
Our novel *in vitro* model of Gaucher's osteoclasts mirrors observations made in studies using osteoclasts derived from the peripheral blood of patients with Gaucher's disease. This system may provide a useful tool for understanding the mechanisms of increased bone turnover in Gaucher's disease and in particular the intracellular consequences of perturbed GBA activity within the osteoclast. This may provide insights for the development of alternative or complementary therapies to prevent the aberrant bone turnover associated with this rare disease.

DOI: 10.1530/endoabs.34.P26

P27

The diagnosis of osteoporosis among South Indian male and female subjects with low impact hip fracture and comparison between Indian Council of Medical Research and Caucasian Bone Mineral Density Databases

Nihal Thomas, Sahana Shetty, Nitin Kapoor, H S Asha, Dukhabandhu Naik, P Visalakshi & Thomas Paul
Christian Medical College, Vellore, India.

Aims and objectives
This study was undertaken to look at the agreement between the hologic database (HD) based on NHANES bone mineral density (BMD) data in Caucasians and the ICMR database (ICMRD) published in 2010 in defining normal and subnormal BMD in subjects with or without hip fracture and to arrive at a BMD cut off which has a high sensitivity of predicting fracture.
Materials and methods
A cross sectional study of 3098 subjects (men-341 and women-2757) (mean age $\pm$ S.D. $=60.1\pm7.6$ years), which included 314 subjects with low impact hip fracture, 2321 from the hospital database and healthy postmenopausal women ($n=461$) from community who underwent (DXA) scanning between 2007 and 2012. Recalculated *T*-scores from ICMRD were used for the diagnosis of osteoporosis and compared with HD. The threshold of hip BMD which best predicts the fracture risk was assessed using ROC curve.
Results
Almost a perfect agreement existed between two databases for the diagnosis of osteoporosis at the hip (Kappa-0.82, $P<0.0001$) in overall subjects and substantial in subjects with hip fracture (Kappa-0.65, $P<0.0001$). Seventy-three of 314 (23.5%) defined as osteoporosis according to HD were classified as osteopenia according to ICMRD. The ROC derived BMD cut-off of 0.681 gm/cm^2 had a 82% sensitivity and a 71% specificity to detect a fracture which corresponded with a *T*-score of -2.1 using a HD and -2.0 with ICMRD using hospital database as controls. When healthy postmenopausal women from community were taken as controls, BMD of 0.681 gm/cm^2 had 82% sensitivity and 71% specificity in predicting fracture risk which corresponded to a *T*-score of -2.1 using a HD and -2.0 with ICMRD.
Conclusions
The threshold of hip BMD *T*-score for treating osteoporosis may have to be redefined if the ICMR reference database is used. This study also highlights the importance of assessing risk factors other than BMD affecting bone health for making therapeutic decisions.

DOI: 10.1530/endoabs.34.P27

P28

Evaluation of a glucocorticoid-induced osteoporosis model using ImageJ

Elda Pacheco-Pantoja, Francisco Arias-Garcia, Ximena Montes-de-Oca, Sergio Hernandez-Rosado, Luis Cab-Cesar, Brahim Rojano-Carrillo & Gustavo Rico-Toro
Escuela de Medicina, Universidad Anahuac Mayab, Merida, Mexico.

Glucocorticoid-induced osteoporosis is the most common form of secondary osteoporosis. The primary effects are on osteoblasts and osteocytes. Glucocorticoids impair the replication, differentiation and function of osteoblasts. These effects lead to a suppression of bone formation, characteristically in the pathogenesis of this form of osteoporosis. On the other hand, available software for image analysis are expensive, inflexible or methodologically dense. ImageJ, an open source, has proven easy to operate, and retrieve reliable results.
We used a commercial dexamethasone, a widely used and potent synthetic member of the glucocorticoid class that has anti-inflammatory and immunosuppressant effects to induce bone loss in male rats Wistar.
Briefly, rats were splitted in three groups: the 'low' dose (5.25 mg/kg), the 'high' dose (8 mg/kg) and the control group (saline solution), which were administered for 5 weeks. The rodents were screened every week for the weight change. At the end of the experiment the animals were eutanized and we obtained blood from cardiac punction for bone alkaline phosphatase and tartrate resistant acid phosphatase (TRAP) determinations. Also we extracted the femurs and fixed for histological image examination using ImageJ software.
The results indicated that alkaline phosphatase was higher in the group with 'high' dose ($P=0.048$) compared with control, but no difference was found between the 'low' dose and control. Also, no significant difference was found in the TRAP circulating levels. The image analysis was focused on the relative quantitation of the cortical bone surface, delimiting the cortical area over the total histological bone section under scrutiny. These data showed significant decreases in both treatment groups ($P<0.01$) when compared with control.
Although it is known that ImageJ has a plugin for bone analysis (BoneJ), the results showed here compel for the use and development of cheaper and more flexible options in order to evaluate and validate osteoporosis animal models.

DOI: 10.1530/endoabs.34.P28

P29

The worlds of primary and secondary hyperparathyroidism often collide; what effect do variable regimens of supplementing vitamin D have in primary hyperparathyroidism?

Antonia Ugur & Emma Ward
Leeds Teaching Hospitals, Leeds, UK.

Introduction
Primary hyperparathyroidism causes accelerated bone turnover and the consensus is to measure and act upon the 25hydroxyvitamin D level in order to reduce the drive to PTH production, slow down BMD loss, and prevent hungry bone syndrome. How best to replace vitamin D is less clear so we chose to audit our current practice.
Method
In 12 months 120 patients with primary hyperparathyroidism were identified retrospectively, 98 of which were vitamin D insufficient (25hydroxyvitamin D <72.5 nmol/l). These were audited as to the action taken and the change in 25hydroxyvitamin D, calcium and PTH.
Results
21.4% of the 98 cases were loaded with the equivalent to 300,000U over 10 weeks, this being the most common decision in the "deplete" subgroup. The majority (44.9%), especially in the subgroup 25-50 nmol/l were given 20,000 to 60,000U monthly. The remaining 33 cases were not given any vitamin D supplementation.
30% of cases referred for surgery were not supplemented but with no detrimental effects.
There was an increment in calcium in all groups except those given <1000U daily (-0.04 mmol/l). The largest mean difference of $+0.13$ mmol/l in the subgroup loaded to 300,000U by weekly doses coincided with the largest mean change in 25-OHvitD level (70.67 nmo/l) without a PTH decrement (mean $+$ 0.86 pmol/l). Those receiving a stat dose of 300,000U did however show reduction in the PTH (mean -2.03 pmol/l), and this was as safe as other regimens for calcium change ($+0.05$ mmol/l), even with influence by an outlying result of $+0.9$ mmol/l. A subgroup of nine patients on 1 g supplement of calcium a day demonstrated a mean rise in calcium of $+0.12$ mmol/l, but despite minimal impact on 25-OHvitD, reduced PTH by a mean of -12.47 pmol/l.
Discussion
There doesn't appear to be one best regimen to use and none appear detrimental. The variable effects on PTH level would warrant further investigation.

DOI: 10.1530/endoabs.34.P29

P30

An ectopic parathyroid adenoma presenting with reduced conscious level and severe hypertension

Rediet Wiebel, Kimberley Lambert & Azraai Nasruddin
University Hospital Southampton, Hampshire, UK.

A 77-year-old lady presented with reduced conscious state, dysphasia and profound confusion on a background of behavioural change and anorexia over the previous 10 days. She was previously independent with history of hypertension controlled on atenolol (100 mg). She was significantly hypertensive 200/100. Her serum calcium was elevated at 4.01 mmol/l (2.05–2.60). The serum parathyroid hormone (PTH) was also significantly elevated at 26 pmol/l (0.5–6.4) consistent with primary hyperparathyroidism. In view of her neurological state, a brain CT scan was performed which did not show any acute abnormality. She was aggressively hydrated with i.v. fluids followed by pamidronate. Her serum calcium improved to 3.29 mmol/l with improvement in drowsiness and blood pressure but with persistent confusion. On day 7 of her admission she was commenced on cinacalcet (30 mg b.d.) while undergoing investigations to localise a parathyroid adenoma. Over the next 1 week her serum calcium gradually normalised and was 2.57 mmol/l on day 15 with significant improvement in her confusion. However, her serum calcium started to rise again to 2.90 mmol/l over the subsequent week requiring increase in cinacalcet dose to 60 mg b.d. which maintained her serum calcium around 2.7 mmol/l but after a further 1 week was again rising above 3 mmol/l. Neck ultrasound failed to locate a parathyroid adenoma. Sestamibi scan suggested a large ectopic parathyroid adenoma within the superior mediastinum which was confirmed on CT imaging. She subsequently underwent urgent surgical removal of a 5.5×2.5 cm parathyroid adenoma via neck dissection with no post-surgical complications and normalisation of serum calcium (2.36 mmol/l) and PTH (3.1 pmol/l) 1 week post-op.
Conclusion
Primary hyperparathyroidism can present with reduced conscious state and profound confusion, requiring urgent treatment. Cinacalcet is a useful agent to manage severe hypercalcaemia, however the effects of cinacalcet as demonstrated in this case may be transient. Accurate pre-operative localisation is particularly important in cases of severe hypercalcaemia as the consequence of failed surgery can be disastrous due to the risk of developing hypercalcaemic crisis.

DOI: 10.1530/endoabs.34.P30

Clinical biochemistry

P31

Effects of light at night and rotating shift on circadian pattern of neuroendocrine chronomolecules

B Anjum[1,2], Narsingh Verma[2], Sandeep Tiwari[3], Ranjana Singh[1] & Abbas Mahdi[1]
[1]Department of Biochemistry, King George's Medical University, Lucknow, Uttar Pradesh, India; [2]Department of Physiology, King George's Medical University, Lucknow, Uttar Pradesh, India; [3]Department of Surgery (Gen), King George's Medical University, Lucknow, Uttar Pradesh, India.

Objectives
The present study investigate the effect of light at night and rotating shift on circadian pattern of salivary cortisol and melatonin sulphate level in night shift workers and to find out whether these changes in the circadian pattern produce by night shift are reversible in due course of time. Sleep loss may be a novel risk factor for various hormones and sleep disturbances leads to metabolic and endocrinal disorders due to interference with diet, circadian metabolic rhythms, and lifestyle.
Method
62 Healthy nursing professional of both sex who perform day and night shifts. Saliva and urine samples were collected at $\sim$8 h interval in their night shift and day shift schedule. Cortisol and melatonin sulphate were estimated by the ELISA method. Groups were compared by applying paired *t*-test.
Results
Evening cortisol level did not show a significant pattern between night (3.22 ± 2.09) vs day shift (2.97 ± 1.76). Extremely significant difference was found in night cortisol levels among night (4.34 ± 3.37) vs day shift (2.70 ± 2.32), ($P<0.001$) due to recovery during day shift. Alteration in mean morning cortisol level was also found between night (3.73 ± 2.47) vs day shift (5.00 ± 2.73). However, this pattern was not highly significant. Night melatonin level was found declined as compared to morning level and this pattern was significant when compared night melatonin between night (16.71 ± 11.98) vs day shift (22.71 ± 13.25) and morning melatonin level between night (20.07 ± 14.13) vs day shifts (28.26 ± 14.14) ($P<0.001$). Altered melatonin levels were found in night and in the morning samples during night shift.
Conclusion
Alterations in circadian pattern of salivary cortisol and melatonin sulfate were found during night shift due to sleep deprivations and internal desynchronization. Sleep loss might be associated with decreased melatonin level leads to endocrinal and cardiovascular diseases.

DOI: 10.1530/endoabs.34.P31

P32

Limitations of dexamethasone suppression tests for Cushing's disease: a reminder!

Emanuela Bejinariu, Shiu-Ching Soo & Ritwik Banerjee
Luton and Dunstable University Hospital, Luton, UK.

Case history
A 70 years old man with longstanding resistant hypertension on five antihypertensive agents, type two diabetes and raised BMI at 35.6 was referred to the endocrinology clinic for exclusion of possible Cushing's disease. Clinically he had truncal obesity with plethoric face but no telangiectasia, easy bruising, purple striae or myopathy.
Investigations
Endocrine examinations revealed normal 24 h urine free cortisol levels on two separate occasions (82 and 152 nmol/l, reference range <270 nmol/l). Overnight dexamethasone suppression test did not suppress the cortisol level (132 nmol/l). Cortisol also failed to suppress after low dose and high dose dexamethasone suppression tests (111 and 253 nmol/l respectively). In-patient midnight cortisol level was 39 nmol/l while 0900 h cortisol was 169 nmol/l showing intact circadian rhythm.

Results and treatment
In conclusion he had normal 24 h urine free cortisol and midnight cortisol but cortisol levels failed to suppress after ODST, LDDST or HDDST. Clinical history identified he was on long-term treatment with phenytoin and phenobarbitone for epilepsy. We believe these two agents are responsible for the failure of dexamethasone suppression in view of their previously well reported enzyme induction effect.
Conclusions and points for discussion
While current antiepileptic agents include lamotrigine, levetiracetam, topiramate, some patients are still well controlled on older agents as phenobarbitone and phenytoin. A good clinical history including drug history is still a necessity before enrolling on complicated dynamic function tests which are not without patient inconvenience and financial burden to a department. Agents that induce CYP3A4 include phenytoin, phenobarbitone, carbamazepine, rifampicin, and alcohol. These are well recognised to produce falsely positive dexamethasone suppression tests by accelerating its clearance. In consequence we only rely on 24 h urine free cortisol levels and midnight cortisol as screening tests for such cases and we need to bear in mind their limitations too.

DOI: 10.1530/endoabs.34.P32

P33

Are we investigating and managing hyponatremia in hospitalised patients properly: a re-audit

Laura Pearson, Ranganatha Rao & Parijat De
Department of Diabetes and Endocrinology, City Hospital, Birmingham, UK.

Aims
To assess the prevalence, investigations and management of hyponatraemia in hospital inpatients (previously deemed inadequate) over a 2-week period (1–15 May 2013) and compare with data collected in 2011, following the introduction of updated clinical guidelines in our trust.
Methods
Hospital notes and electronic records of all patients with a sodium level of <130 mmol/l were analysed.
Results
43/255 (17%) patients had sodium <130 mmol/l. Complete data was available in 35/43 (78%) – 24 males, 11 females, average age 69 years, mean sodium 128 mmol/l, 7 (3%) had sodium <125 mmol/l.
Hydration status was mentioned at diagnosis in 21 (60%) patients: 13 (37%) were euvolaemic, 5 (14%) hypovolaemic and 3 (9%) hypervolaemic. Further investigations were only requested in 5 (14%) patients. No patients were diagnosed with SIADH, but from the results, 2 (6%) patients had findings consistent with SIADH.
5 (14%) patients were put on fluid restriction, 13 (37%) received i.v. fluids, 6 (17%) of patients had diuretics withheld. Demeclocycline was not prescribed in any case. 11 (13%) required i.v. antibiotics and 3 (9%) were admitted to ITU. Endocrinology review was not requested in any case.
4 (11%) patients died and 16 (46%) were discharged with a sodium <130 mmol/l. The average length of hospital stay was 14.5 days (range 1–61 days).
Compared to the previous audit, hyponatraemia was mentioned in the diagnosis in fewer cases (14 vs 26%), as was hydration status (60 vs 67%). However, investigations for SIADH were requested in twice as many (14 vs 7%) and more patients had active management (fluid restriction and withholding of diuretics).
Conclusion
Investigation of hyponatraemia has improved following introduction of new local guidelines, although management is still suboptimal. Endocrinologists continue to be underutilised. Our plan is to disseminate hyponatremia guidelines more widely and incorporate hyponatremia management within the core teaching programme of junior doctors.

DOI: 10.1530/endoabs.34.P33

P34

A routine combined LC–MS/MS assay for male androgens

Laura Owen[1,2] & Brian Keevil[1,2]
[1]UHSM, Manchester, UK; [2]MAHSC, Manchester, UK.

Introduction
The measurement of male androgens in most NHS laboratories is often limited to testosterone alone. To more accurately determine the androgen status in men the measurement of other androgens such as DHT and DHEA would be beneficial however these are difficult to measure without derivatisation. We report a combined LC–MS/MS assay for the measurement of testosterone, androstenendione, DHT and DHEA on a small sample volume.
Methods
Zinc sulphate (100 µl) was added to 100 µl of sample. After mixing, acetonitrile containing internal standards was added and was further mixed. The samples were centrifuged before analysis. Samples were extracted using an automated on-line solid phase extraction on a C18 cartridge by a Waters Acquity/OSM and analytes were measure using a Waters TQS tandem mass spectrometer.
Results
Chromatographic separation was achieved between all four androgens. The run time was 6.2 min per sample. The lower limit of quantitation was 0.2 nmol/l for testosterone, 0.3 nmol/l for androstenedione, 0.17 nmol/l for DHT and 2 nmol/l for DHEA. The CV of the assay for testosterone and DHT was $<6\%$, androstenedione was $<9\%$ and DHEA was $<5\%$. The testosterone and androstenedione gave the following comparisions with the routine LC–MS/MS assay: Testosterone (combined) $= 1.01 \times$ existing assay $+ 0.07$ nmol/l and androstenedione (combined) $= 1.09 \times$ existing assay $- 0.29$ nmol/l.
Discussion
We have developed a rapid assay for the LC–MS/MS measurement of testosterone, androstenedione, DHT and DHEA in a routine clinical laboratory. The assay requires a small volume of sample and all analytes are measured simultaneously without derivitisation achieved by using a high sensitivity mass spectrometer. The assay is rapid and simple to prepare and has the potential for routine clinical use or for the analysis of large numbers of samples involved in clinical trials.

DOI: 10.1530/endoabs.34.P34

P35

Urinary kisspeptin as a novel marker of pregnancy

Alexander Comninos, Channa Jayasena, Shakunthala Narayanaswamy, Ali Abbara, Gurjinder Nijher, Mansimran Cheema, Zainab Malik, Mohammad Ghatei, Stephen Bloom & Waljit Dhillo
Imperial College London, London, UK.

Background
Kisspeptin is an RF amide peptide hormone critical for reproductive function. Kisspeptin is also abundantly expressed in the placenta, where it has an important physiological role in regulating placental invasion. Accordingly, plasma kisspeptin levels rise dramatically during normal pregnancy. Lower plasma levels of kisspeptin are associated with poor pregnancy outcomes such as recurrent miscarriage, intrauterine growth restriction and pre-eclampsia. Urinary measurement of kisspeptin may provide a novel and practical tool for screening patients for major obstetric complications. However, it is not currently known whether kisspeptin can be detected and quantified in the urine of pregnant women.
Aim
To determine the clinical utility of urinary kisspeptin measurement in healthy pregnant women.
Methods
Forty-nine healthy third trimester pregnant women (gestational age 34 ± 0.6 weeks) from a single maternity unit and 50 healthy non-pregnant women were recruited. Urine and blood were simultaneously collected from all volunteers in plain containers and lithium heparin tubes respectively, each containing 5000 kallikrein inhibitor units of aprotinin. Kisspeptin levels were determined by in-house manual RIA and urine creatinine by kinetic alkaline picrate method.
Results
Mean levels of plasma kisspeptin were over 200-fold greater in third trimester pregnant women compared with non-pregnant women ($13\,783 \pm 864$ pmol/l, pregnant; 65 ± 13 pmol/l non-pregnant, $P<0.0001$). Mean levels of urine kisspeptin were greater in pregnant women when compared with non-pregnant women (301 ± 59 pmol/l, pregnant; 80 ± 19 pmol/l, non-pregnant, $P<0.001$). Urine kisspeptin levels were significantly correlated with plasma kisspeptin levels in pregnant women ($r=0.35$, $P<0.01$). The urine kisspeptin:creatinine ratio was also significantly greater in pregnant women compared with non-pregnant (37 ± 6 pmol/umol, pregnant; 7 ± 1 pmol/umol, non-pregnant, $P<0.0001$).
Conclusion
We demonstrate for the first time that kisspeptin levels are elevated in urine during pregnancy. Urine collection may therefore offer a non-invasive and simple method of screening for pregnancy and obstetric complications, which is particularly suited to the busy clinical setting of the obstetric clinic.

DOI: 10.1530/endoabs.34.P35

P36

The effects of climate on the incidence of thiazide diuretic induced hyponatraemia in the UK

Christopher Redford, Thomas Skinner, Andrzej Rys & Jamie Smith
South Devon Healthcare Foundation Trust, Torbay, UK.

Although TIH is a well-established complication of this drug class, the extent of this problem and the serious morbidity that can result is often not appreciated. At our centre, we became aware of three serious cases of TIH over a 2-week period during the 'heat wave' in July 2013. In at least one of these cases excessive water drinking was a clear precipitant. We therefore determined the incidence of TIH at our hospital and in particular whether there is a seasonal effect on admission rates. We retrospectively reviewed records of all admissions to our hospital containing a discharge diagnosis code of hypo-osmolality/hyponatraemia over a 14-month period. The medical discharge summary of each case was reviewed to identify cases where TIH was implicated as the predominant cause. Patients with a co-morbidity of heart failure, malignancy or liver disease were excluded from the analysis. Average monthly temperature data were obtained from public records (Met Office).

443 patients were admitted with a coding diagnosis of hypo-osmolality/hyponatraemia between June 2012 and August 2013. Amongst these there were 73 cases of TIH. Cases of TIH were sorted chronologically by month of admission. The monthly average temperature was then compared with the monthly TIH admission rate. Our data demonstrate a background monthly admission rate of three to four cases of TIH but with evidence of seasonal peaks in the hotter months with nine and seven cases admitted in July and August 2012 respectively and ten cases in July 2013.

TIH is common cause for acute medical admission in the UK. Our data suggest evidence of seasonal variation in the incidence of the problem with patients being at greater risk of developing this complication during the hotter months of the year. An increase in fluid intake during hotter weather may underlie this association.

DOI: 10.1530/endoabs.34.P36

P37

Generation of a long acting GCSF for treatment of neutropenia and stem cell harvest

Abdulrahman Alshehri, Richard Ross & Ian Wilkinson
The University of Sheffield, Sheffield, UK.

Rationale

Over the last 20 years, granulocyte colony-stimulating factor (GCSF) has become a recognized therapy in the treatment of patients suffering from neutropenia. Current therapies require daily injections of GCSF to stimulate stem cell production and response to treatment is often unpredictable as GCSF is rapidly cleared. A number of approaches to reducing GCSF clearance have been tried mainly through conjugation with another moiety. The technologies already being employed, included PEGylation, immunoglobulins and glycosylation to increase the half-life of rhG-CSF, however although these approaches have reduced clearance the pharmacokinetic profile has remained unpredictable. The aim of this project is to create a long acting GCSF with predictable pharmacokinetic profile to provide a more effective treatment for generating stem cells for bone marrow transplantation.

Hypothesis

The incorporation of variable glycosylated linkers between two GCSF molecules will create a construct with high molecular weight and protected from proteolysis resulting in reduced clearance while retaining bioactivity.

Methodology

GCSF tandem molecules with linkers containing between two and eight NAT glycosylation motifs and their respective controls (Q replaces N in the sequence motif NAT) were cloned, and sequenced. Following expression in Chinese hamster ovary (CHO) cells, expressed protein was quantified by ELISA and analysed by SDS–PAGE to confirm molecular weights. *In vitro* bioactivity was tested using an AML-193 proliferation assay. Immobilised metal affinity chromatography (IMAC) was used to purify the protein.

Results

GCSF tandem molecules with a flexible $(Gly_4Ser)_n$ linker and glycosylation sites were expressed in CHO cells. The degree of glycosylation correlated with an increase in molecular weight, with the addition of 2, 4 and 8 glycosylation motifs, reaching a peak MW of around 52, 60 and 70 kDa compared to 45.4, 45.4 and 48.5 kDa for non-glycosylated controls respectively. Bioactivity was confirmed for all glycosylated tandems and un-glycosylated controls comparable to native GCSF.

Conclusion

These results demonstrate that the use of glycosylated linkers to generate protein-tandems results in glycosylation and an increased molecular weight whilst retaining bioactivity. Future studies are required to investigate stability and *in vivo* studies to test protein clearance.

DOI: 10.1530/endoabs.34.P37

P38

Hypercalcaemia referrals from primary care: a retrospective audit

Negar Maghsoodi, Caje Moniz & Royce Vincent
Clinical Biochemistry, London, UK.

Background

Primary hyperparathyroidism (PHP) is the commonest cause of hypercalcaemia, with an annual incidence rate (AIR) of 4/100 000 and peak age incidence of 50–60 years. Our hospital serves a population with age $\geq$18 of around 500 000. A corrected calcium (cCa) $\geq$3.00 mmol/l is a critical phoning limit in our biochemistry laboratory.

Aim

To assess the incidence of hypercalcaemia in the community, the referral pattern of hypercalcaemia in the primary care and the laboratory practice on phoning out results.

Methods

This retrospective audit included bone profiles (cCa, phosphate and alkaline phosphatase) from primary care between July 2010 and November 2012. Those with cCa $<$2.8 mmol/l, aged $<$18 years and known to have hypercalcaemia previously were excluded.

Results

Overall 22 patients had cCa $\geq$2.8 mmol/l (19F) aged 59 (36–82) years. 68% ($n=15$) were referred specifically for hypercalcaemia, 23% ($n=5$) for other reasons and 9% ($n=2$) were not referred. 32% had PHP, 18% were on Ca/vitamin D supplements, 14% had malignancies and 36% had other causes (including secondary hyperparathyroidism) or no known cause. Vitamin D was requested in 50% and parathyroid hormone in 60% but none had a urine calcium request. All cCA $\geq$3.00 mmol/l were phoned by the laboratory. Our study led to referral and diagnosis of a patient with PHP who was not investigated since 2010.

Conclusions

The AIR of hypercalcaemia was 1.9/100,000. There is a wide variation in the referral practice for hypercalcaemia in the community. A guideline on hypercalcaemia management for the primary care may improve patient outcome.

DOI: 10.1530/endoabs.34.P38

P39

Development of a LC–MS/MS method for the measurement of serum 17-hydroxyprogesterone during the follicular and luteal phases of the menstrual cycle

Charlotte Hill & Andrew Davison
Department of Clinical Biochemistry and Metabolic Medicine, Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, UK.

Aims

Develop and validate a LC–MS/MS assay for the measurement of serum 17-hydroxyprogesterone (17-OHP) and to establish reference ranges for levels observed in the follicular and luteal phases of the menstrual cycle.

Method

Serum samples (200 µl) underwent liquid extraction using di-ethyl ether (1 ml). A deuterated internal standard was used. 20 µl extract was injected onto a Waters Atlantis C18 column using a Waters 2795 Alliance HPLC separations module coupled to a Waters Premier tandem mass spectrometer. Samples were also analysed using RIA (Coat-A-Count, DPC). Serum samples were obtained from patients during the follicular phase of the menstrual cycle (days 1–3) and luteal phase (day 21).

Results

Measurement of 17-OHP was linear to 100 nmol/l ($R^2=0.99$), lower limit of quantitation was determined as 0.3 nmol/l and inter-assay CV were $<$20%. Correlation with RIA was poor with results up to 40% lower by LC–MS/MS (mean bias 7 nmol/l). Comparison with other LC–MS/MS methods (UKNEQAS data) was good ($y=1.02x+1.54$, $R^2=0.98$). No interference was observed from 11-deoxycortisol or 21-deoxycortisol. Mean ($\pm$S.D.) concentrations of 17-OHP in the follicular phase ($n=40$) were 0.75 nmol/l (0.38), giving a reference range of 0.3–1.50 nmol/l. Mean concentrations in the luteal phase ($n=39$) were 2.7 nmol/l (1.59) giving a reference range of 1.2–4.4 nmol/l.

Conclusions
A method has been developed for the measurement of and 17-OHP. Reference ranges have been established for 17-OHP in the follicular and luteal phases of the menstrual cycle.

DOI: 10.1530/endoabs.34.P39

P40

Audit on short synacthen test: are 30 and 60 min samples necessary?

Yasmeen Khalid, Edward Kearney & Stonny Joseph
Queen Elizabeth The Queen Mother Hospital, Margate, Kent, UK.

Background
Short synacthen test is used to assess adrenal function by injecting 250 μg of synacthen (tetracosactide) and measuring cortisol at baseline and subsequently after 30 and 60 min of the injection. In our hospital the cut-off for a normal test is a peak cortisol of 480 nmol/l or an increment of 200 or more from the baseline value.
Methodology
We reviewed the results of 50 short synacthen tests performed in our hospital within the last year to see how many patients had a normal result based on the above stated criteria. Cortisol was measured using Abbot Diagnostics.
Results
50 patients underwent the test, 35 were females and 15 were men (mean age 56 years). Out of a total of 50 tests performed only 6 (12%) were abnormal and for all six, the peak value was <480 and the total rise in cortisol was <200.
44 patients had a normal short synacthen test result. Of these 42 (95%) patients had a 30 min cortisol of ≥ 480 nmol/l or an increment of 200 from the baseline. In the remaining two cases the 30 min value was <480 and the total increment in cortisol was <200. However for both these cases the 60 min result was >480.
Discussion and conclusion
The protocol for short synacthen tests and the cut-off values for normal response varies in different hospitals. In our hospital we currently check a 30 and 60 min sample after synacthen. Our audit data shows that measuring the baseline and 30 min sample will pick 95% of the positive results and 100% of the negative results. Similarly a baseline cortisol and 60 min sample will pick all the positive and all the negative results.

DOI: 10.1530/endoabs.34.P40

P41

A comparison of calculated bioavailable testosterone with calculated free testosterone

David Tesh, Timothy Davies, Miles Levy & Trevor Howlett
University Hospitals of Leicester NHS Trust, Leicestershire, UK.

Objective
Biochemical assessment of male hypogonadism relies on estimation of freely available testosterone. Gold standard measurement is by equilibrium dialysis but this is not practical in clinical use. We compared two calculation methods; bioavailable (non-SHBG bound) testosterone (Morris *et al.*), and free (non-SHBG non-albumin bound) testosterone (Vermeulen *et al.*) for their diagnostic performance.
Design
Free testosterone and bioavailable testosterone were assessed for agreement for a cohort of 301 males with symptoms of hypogonadism.
Methods
Total testosterone was measured by chemiluminescence competitive binding assay by Siemens. Sex hormone binding globulin (SHBG) was measured by chemiluminescence double antibody assay by Siemens. Free testosterone was calculated by the method of Vermeulen *et al.*, and bioavailable testosterone by the method of Morris *et al.* Regression analysis was used to compare the two.
Results
We found that the Vermeulen free $T=0.174$ nmol/l corresponds to Morris bioavailable of 2.7 nmol/l. The correlation between the two methods is good ($R^2=0.92$).
Conclusions
For patients with symptoms of hypogonadism in our area there is no significant difference in diagnostic performance between bioavailable and free testosterone as calculated by the above methods.

DOI: 10.1530/endoabs.34.P41

P42

Urinary 3-methoxytyramine as a biomarker of phaeochromocytoma and paraganglioma tumours

Christina Neophytou, Sarah Darch, Jasdeep Singh Gill, Maggie Hancock, Karim Meeran, Fausto Palazzo & Tricia Tan
Imperial College, London, UK.

Phaeochromocytomas (PCC) and paragangliomas (PGL) are rare tumours derived from the sympathetic or parasympathetic paraganglia. They characteristically secrete catecholamines (noradrenaline/adrenaline/dopamine), which are metabolised to the metanephrines (normetadrenaline/metadrenaline/3-MT respectively). These tumour markers can be detected in acidified 24-h urine collections as first-line investigative tests. Plasma 3-MT has been characterised as a biomarker of metastatic PGL/PCC. In the present study, we aim to characterise the diagnostic utility of urine 3-MT.
Objectives
To evaluate the diagnostic utility of urine 3-methoxytyramine (3-MT) in the diagnosis of metastatic PCC and PGL disease.
Design and setting
A retrospective study using 44 patients with no known PCC or PGL disease (34 being screened for hypertension, ten with previous disease which was completely resected) compared to 21 patients with known PCC or PGL disease (nine head and neck, nine abdominal including PCC, three thoracic). Those patients with PCC/PGL were further classified into those with metastatic (7) and without metastatic disease (14). Acidified 24 h urine samples were assessed for 3-MT levels using an in-house HPLC method.
Results
ROC curve analysis of 3-MT excretion for the diagnosis of PCC/PGL showed that the area under curve (AUC) was 0.8226. Mean ($\pm$S.D.) levels of 3-MT in those with PCC/PGL was 3.83 ± 7.08 μmol/24 h and in those without 0.82 ± 0.31 μmol/24 h ($P<0.0001$, Mann–Whitney U test). Comparing those with metastatic disease to those without, the ROC-AUC was 0.8367. Mean ($\pm$S.D.) levels of 3-MT in metastatic disease was 8.99 ± 10.96 μmol/24 h and without metastatic disease 1.25 ± 0.52 μmol/24 h ($P=0.0153$, Mann–Whitney U test).
Conclusions
3-MT is a good biomarker for the diagnosis of PCC or PGL disease, and particularly for distinguishing metastatic vs non-metastatic disease.

DOI: 10.1530/endoabs.34.P42

P43

Evaluation of the efficacy of transdermal delivery of chloroquine on *Plasmodium berghei*-infected male Sprague–Dawley rats: effects on blood glucose and renal electrolyte handling

Happiness Sibiya, Metse Serumula & Cephas T Musabayane
University of KwaZulu-Natal, Durban, South Africa.

Oral administration of chloroquine (CHQ) evokes adverse effects on glucose homeostasis and kidney function possibly due to transiently high plasma CHQ concentration or malaria. We have, however, reported that transdermally administered CHQ via the pectin CHQ-matrix patch formulation sustains controlled CHQ release into the bloodstream in experimental animals. Accordingly, the current study was designed to compare the ability of oral and transdermal CHQ treatments to clear parasites of *P. berghei* infected rats. The other objective was to distinguish between the effects of malaria and CHQ treatments on blood glucose, blood pressure and kidney function. To prepare the patch, pectin was dissolved in deionized water followed by adding CHQ, DMSO and antioxidants. After freezing, $CaCl_2$ was added for cross-linking and patch formation. Parasitaemia, blood glucose and renal function were monitored in separate groups of non-infected and *P. berghei*-infected rats treated twice daily with oral CHQ (30 mg) or once off topical application of the pectin-CHQ matrix patch (28 mg) over a 21-day period divided into days of pre-treatment (0–7), treatment (8–12) and post-treatment (13–21). Oral CHQ treatment significantly decreased blood glucose concentrations of both the non-infected and infected animals. By day 5 oral CHQ treatment, blood glucose concentrations of non-infected and infected rats were reduced to values ranging from 2.93 to 3.03 mmol/l whilst transdermal treatment had no significant effects on blood glucose levels (5.44 ± 0.31 mmol/l). On the other hand, oral and transdermal treatments equally reduced *P. berghei* parasites to levels that were undetectable by day 5. Oral CHQ treatment increased urinary Na^+ and K^+ excretion of non-infected and *P. berghei*-infected rats whilst the pectin-CHQ matrix patch did not influence these parameters. We conclude that the pectin-CHQ matrix patch has the potential avert the adverse effects that are associated with oral administration of CHQ.

DOI: 10.1530/endoabs.34.P43

P44

Measurement of dexamethasone by LC–MS/MS after a 1 mg overnight dexamethasone suppression test

Miguel Debono[1], John Newell-Price[1], James Hawley[2] & Brian Keevil[2]
[1]University of Sheffield, Sheffield, UK; [2]University Hospital of South Manchester, Manchester, UK.

Background
The overnight dexamethasone (DEX) suppression test is useful for the investigation of hypercortisolism, however several factors may influence its performance. Intestinal uptake of DEX, inactivation by conversion by CYP3A4 in the liver and renal clearance can all affect test variability. It is also known that several drugs can either reduce or accelerate CYP3A4 activity, thereby affecting blood DEX concentrations. Interpretation of the test would be greatly enhanced by knowledge of the plasma DEX concentration when the morning cortisol sample is taken. We describe a sensitive LC–MS/MS assay for the analysis of the low concentrations of DEX encountered during this test.
Methods
Samples were obtained at 0800–0900 h from 90 postmenopausal women who all had a 1 mg ONDST. 250 µl sample and 10 µl internal standard (d4-DEX) was extracted with 1 ml MTBE. After mixing, the supernatant was blown down and reconstituted in 80 µl 40% MeOH. Sample extract was injected onto a C18 column and the eluate measured on a Xevo TQ mass spectrometer. Dexamethasone was eluted with a linear methanolic gradient. Total run time was 2.5 min.
Results
The LLOQ was 0.5 nmol/l and recovery was 100% (range 98–103%). CV was $<8\%$ at 1.9 nmol/l. The mean (S.D.) dexamethasone level was 7.8 (3.6) nmol/l whilst the mean dexamethasone cortisol was 31 nmol/l (S.D. 33 nmol/l; range 11–317 nmol/l). 6/90 patients had a dexamethasone cortisol level of >50 nmol/l and 4/6 of these participants had dexamethasone levels <5.6 nmol/l.
Discussion
We have developed a highly sensitive method for the evaluation of DEX in morning serum samples after an ONDST. 67% of participants who failed the ONDST had low dexamethasone levels highlighting the possibility of a false positive test. The study suggests that simultaneous measurement of dexamethasone and cortisol may allow more accurate evaluation of dexamethasone suppression test results but greater numbers are needed to define precise dexamethasone cut-offs.

DOI: 10.1530/endoabs.34.P44

P45

Serum 5HIAA: a better biomarker than urine for detecting and monitoring neuroendocrine tumours?

Joanne Adaway[1,2], Rebecca Dobson[4], Jennifer Walsh[3], Daniel Cuthbertson[4], Philip Monaghan[5], Juan Valle[2,5], Neil Devlin[5] & Brian Keevil[1,2]
[1]Wythenshawe Hospital, Manchester, UK; [2]Manchester Academic Health Science Centre, Manchester, UK; [3]Sheffield Teaching Hospitals NHS Trust, Sheffield, UK; [4]Aintree University Hospital NHS Trust, Liverpool, UK; [5]Christie Hospital NHS Foundation Trust, Manchester, UK.

Background
5-hydroxyindole acetic acid (5-HIAA) (a metabolite of serotonin) is used as a marker for patients with serotonin-secreting neuroendocrine tumours. Currently, most laboratories measure 24 h 5-HIAA excretion in urine samples. Urine collections are cumbersome for the patient and impact on their daily activities; they are consequently often poorly performed, leading to over- or under-collection of urine and inaccurate 5-HIAA excretion results. Furthermore, large volumes of urine present a health and safety challenge to processing laboratories. We compared urinary and serum 5-HIAA using previously published LC–MS/MS methods to assess whether serum 5-HIAA could replace urinary 5-HIAA estimation in the diagnosis and monitoring of neuroendocrine tumours.
Methods
We measured 5-HIAA in 177 paired serum and urine samples from healthy volunteers and patients with known 5-HIAA secreting neuroendocrine tumours.
Results
The results were expressed as a percentage of the reference range and linear regression showed a correlation coefficient of 0.67 with a Passing Bablock regression equation of Serum 5-HIAA $=1.61\times$urine 5-HIAA-0.32. Bland Altman analysis showed a proportional positive bias in the serum 5-HIAA results, with the bias increasing with the 5-HIAA concentration.
Conclusion
We have demonstrated a strong correlation between serum and urine 5-HIAA results. In patients with 5-HIAA concentrations above the reference range, the increase in serum 5-HIAA is greater than that of urinary 5-HIAA, indicating that plasma 5-HIAA may be preferable in the diagnosis and monitoring of neuroendocrine tumours. Furthermore serum 5-HIAA measurement offers greater patient convenience, and may be a better marker than urinary 5-HIAA excretion.

DOI: 10.1530/endoabs.34.P45

P46

High testosterone? Look again!

Tarig Babiker, Mandy Perry, Timothy McDonald, Antonia Brooke & John O'Connor
Royal Devon and Exeter Hospital, Exeter, Devon, UK.

Three premenopausal patients presented with high isolated testosterone without symptoms of androgen excess or illicit drug use.
Case 1
A 22-year-old female presented with daily vaginal bleeds having been on depot injections of progesterone with supplementary norethisterone. Testosterone was 14.5 nmol/l with suppressed gonadotrophins (LH <0.1 IU/l, FSH 0.2 IU/l, oestradiol <19 pmol/l). Her norethisterone was stopped. A repeat biochemical profile after 3 weeks was normal (testosterone 0.8 nmol/l, FSH 4.1 IU/l, LH 3.4 IU/l, oestradiol 230 pmol/l).
Case 2
A 43-year-old female presented with a 3 week history of prolonged menstrual bleeding. She commenced norethisterone; 2 days later testosterone was 7.6 nmol/l, SHBG 66.9 nmol/l, free androgen index 11.36, LH 0.4 IU/l, FSH 1.2 IU/l, oestradiol 198 pmol/l. Repeat testing 12 days after stopping norethisterone was normal: testosterone 0.8 nmol/l, 85.4 nmol/l, free androgen index 0.94, FSH 6.3 IU/l, LH 5.5 IU/l, oestradiol 839 pmol/l, cortisol 302 nmol/l).
Case 3
A 20-year-old female with dysmenorrhoea on the oral contraceptive pill commenced norethisterone with resolution of her symptoms and amenorrhoea. She took lamotrigine for epilepsy but denied other medications. Initial testosterone was elevated: 7.7 nmol/l, SHBG 9.4 nmol/l, free androgen index 81.91, FSH 1.3 IU/l, LH <0.1 IU/l. After 3 weeks, her biochemistry had improved: 17OHP <1 nmol/l, cortisol 169 pmol/l, FSH 0.6 IU/l, LH <0.1 IU/l, testosterone 4.2 nmol/l, SHBG 10.1 nmol/l, free androgen index 41.58. After a further 2 weeks her testosterone was normal (0.9 nmol/l).
All patients had elevated testosterone associated with norethisterone use, which normalised 19–41 days after norethisterone was stopped. Norethisterone is metabolised to 19-norandrosterone (as is nandrolone) via 5-α-reductase but has only weak androgenic activity. We hypothesise that 19-norandrosterone interferes the Roche Modular E170 Testosterone II assay used, giving a falsely elevated testosterone.

DOI: 10.1530/endoabs.34.P46

P47

Vitamin D status in patients visiting a large teaching hospital

Shaina Hirany, Mihail Firan & Ibrahim Hashim
Parkland Memorial Hospital and UT Southwestern Medical Center, Dallas, Texas, USA.

Growing interest in the role of vitamin D in many disease conditions as well as wide availability of vitamin D assays has led to a marked increase in its measurement. Although there are multiple circulating metabolites of vitamin D, those of interest are 25-hydroxyvitamin D2 and D3 and measurements of their relative concentrations is considered helpful. We reviewed vitamin D levels (D2 and D3) obtained on our patient population visiting a large teaching hospital.
Methodology
12 months data for total 25-hydroxy vitamin D, D2 and D3 levels were obtained from patients seen at our hospital. Vitamin D2 and D3 levels had been performed using LC–MS/MS.
Results
A total of 6246 vitamin D measurements from 5515 patients were analysed over a 12 months period. Total vitamin D levels ranged from 6 to 83 (median: 25 ng/ml), vitamin D2 ranged from <4 to 76 (median: 17 ng/ml) and D3 ranged from 2.1 to 58 (median: 13 ng/ml). Only 47% of patients had optimal vitamin D levels. Among patients with vitamin D deficiency, the majority, 92.1% had un-measurable or low D2 levels compared with 73.9% for D3. D2 levels

correlated ($r=0.65$) better with total vitamin D levels when compared with D3 levels ($r=0.51$). Few adult patients ($n=10$) had significant circulating 3-epi-5-OH vitamin D3 levels causing falsely elevated (by 30–79%) total vitamin D levels resulting in a change in vitamin D status from being optimal to exhibiting insufficiency in three patients.
Conclusion
A significant number of patients had suboptimal vitamin D levels. Furthermore, despite dietary fortification, D2 was the most deficient form and was undetectable in a significant number of patients. Measurement of total vitamin D predicted vitamin D status (both D2 and D3). However, measurement of D2 and D3 levels may be needed as second-line investigation and to monitor supplementation.

DOI: 10.1530/endoabs.34.P47

P48

Development of a whole blood assay for the LC–MS/MS measurement of 5-hydroxyindole acetic acid

Suzanne Armitage, Joanne Adaway & Brian Keevil
University of South Manchester, Manchester, UK.

Background
5-hydroxyindole acetic acid (5-HIAA), a metabolite of serotonin, is used as a marker for patients with serotonin-secreting neuroendocrine tumours (NET). Currently, the majority of laboratories measure 5-HIAA excretion in 24 h urine samples. Given the practicality and analytic problems of these samples, our laboratory successfully developed a LC–MS/MS method for the analysis of 5-HIAA in serum samples. Further to this, we have now developed a method to measure 5-HIAA in whole blood samples.
Methods
We developed a method for measurement of 5-HIAA in whole blood samples using a simple protein precipitation step prior to LC–MS/MS analysis. To validate the method, ion suppression, recovery from spiked whole blood, linearity, inter- and intra-assay imprecision and lower limit of quantitation (LLOQ) was assessed. The analysis was performed in positive ion mode using a Waters Quattro Premier; the ion transitions were *m/z* 192.9 > 145.9 and 199.1 > 149.8 for 5-HIAA and d5-5-HIAA respectively.
Results
Using post-column infusion of 5-HIAA, ion suppression was deemed to be negligible. Mean recovery was 93% (range 85–101%), and the method was linear up to 100 000 nmol/l. Within batch and between batch imprecision (CV), assessed over the normal and pathological range, was <9.1 and $<7\%$ respectively. LLOQ was 12.5 nmol/l.
Conclusion
We have successfully developed and validated a method for the measurement of 5-HIAA in whole blood samples. It is proposed that this method could be further developed to enable measurement of 5-HIAA in fingerprick or dried blood samples.

DOI: 10.1530/endoabs.34.P48

P49

Do we need method specific cortisol cut off limits for dynamic function tests?

Kate Braha, Suzannah Phillips, John Dutton & Anna Milan
Royal Liverpool and Broadgreen University Hospital Trust, Liverpool, UK.

Measurement of cortisol is used in the differential diagnosis of Addisons' disease (deficiency), Cushing's disease (excess), hypopituitarism, adrenal hyperplasia and carcinoma. Cortisol is routinely measured by immunoassay, which has wide inter-assay variability with manufacturer dependent bias. Despite this, universal cut-offs are used as criteria for interpretation of dynamic function tests of the pituitary adrenal axis.
Using a fully validated LC–MS/MS method, comparison between the Roche Elecsys immunoassay has been performed. Additionally, synacthen stimulation tests (SST) and overnight dexamethasone suppression tests (DST) have been analysed on both methods. Cortisol levels were on average 25% lower by LC–MS/MS, compared with the immunoassay. Five out of 62 SST ($\sim 8\%$) which satisfied criteria (>550 nmol/l at 30 min) by immunoassay, failed on the LC–MS/MS method and did not reach 550 nmol/l at 60 min. An additional four SST which reached >550 nmol/l at 30 min by immunoassay, did not measure >550 nmol/l until 60 min on LC–MS/MS. 11% of SSTs failed to response to synacthen on both methods. Preliminary data has shown no difference between assays for the DST. However, with the proven positive bias of the immunoassay, some DST that fail to suppress may show adequate suppression when analysed by LC–MS/MS.
In conclusion, interpretation of cortisol dynamic function tests should consider the method of analysis and local guidance should be provided.

DOI: 10.1530/endoabs.34.P49

P50

Reference ranges for salivary steroid measurement during short synacthen tests

Rohan Shotton[1], Ilias Perogamvros[1], Brian Keevil[2], Philip Monaghan[1], Peter Trainer[1] & Claire Higham[1]
[1]Christie Hospital NHS Foundation Trust, Manchester, UK; [2]University Hospital of South Manchester NHS Foundation Trust, Manchester, UK.

Background
The use of salivary cortisol is well validated in the diagnosis of Cushing's syndrome but routine use of salivary measurements during dynamic tests of HPA axis sufficiency is uncommon and reference ranges are not established. Salivary cortisol (SalF) and cortisone (SalE) measurements by LC–MS have several advantages including ease of collection and more accurate reflection of unbound serum cortisol levels in conditions of altered CBG levels (e.g. oestrogen replacement). SalE may have advantages over SalF although this remains to be confirmed.
Method
A retrospective analysis was carried out of the SST results from patients tested at the Christie Hospital NHS Foundation Trust since September 2010. Some data from a previous study were also included. Patients were subdivided by their use of oestrogen-containing medications. SalF and SalE values from 30 to 60 min post-synacthen (250 μg) were plotted against SerF in order to derive reference ranges. Salivary values corresponding to a SerF of 450 nmol/l were calculated using a $y=mx+c$ equation.
Results
Matched serum and salivary values at 30 and 60 min were available from 71 SSTs. Using a cut-off of 450 nmol/l for SerF at 30 min; SalF = 14.3 nmol/l ($n=64$) and SalE = 40.7 nmol/l ($n=16$). Using the same 450 nmol/l cut-off at 60 min; SalF = 22.3 nmol/l ($n=63$) and SalE = 50.6 nmol/l ($n=16$). The use of oestrogen did not significantly affect salivary measurements at either time point.
Conclusions
Novel 30- and 60-min cut offs for salivary SST results are presented, which now require further validation in clinical practice. The use of oestrogen did not lead to significant differences in salivary cortisol or cortisone values. It is important to develop robust reference ranges for steroid measurement by LC–MS, particularly in saliva, as LC–MS steroid measurement is expected to become mandatory in view of the shortfalls of steroid immunoassays.

DOI: 10.1530/endoabs.34.P50

P51

Hyponatraemia prior to discharge from hospital after a general medical admission is associated with a significantly increased risk of readmission within 28 days

Pratik Solanki[1], Benjamin Whitelaw[1], Christine Leong[1,2], John Miell[1,2] & Simon Aylwin[1]
[1]King's College Hospital, London, UK; [2]Lewisham University Hospital, London, UK.

Hyponatraemia is associated with adverse outcomes including increased mortality and risk of falls. It is not previously known whether hyponatraemia, on discharge from hospital, is associated with an increased risk of readmission.
We conducted a retrospective cohort study identifying all patients admitted to a UK teaching hospital as emergency general medical admissions over a 2-month

period. We identified all readmissions within 28 days of discharge and collected data on diagnosis, drug treatment, admission, nadir and discharge serum sodium. Hyponatraemia was defined as [Na] $<$135 mmol/l. Patients who died during the initial admission or who remained in hospital were excluded from the study.
Results
1527 patients were included in the dataset. 26% (397/1527) of patients demonstrated hyponatraemia at some point during their admission and 9% (141/1527) were hyponatraemic at discharge. 238 (16%) patients were readmitted within 28 days. Hyponatraemia at discharge (Na $<$ 135 mmol/l) was associated with an increased risk of re-admission, odds ratio 1.7 ($P=0.0155$). Moderate or severe hyponatraemia (Na $<$ 130 mmol/l) showed a stronger association with readmission: odds ratio 3.4 ($P=0.007$). The prevalence of congestive cardiac failure and the use of anticonvulsant medication were both significantly higher in the re-admitted group (16 vs 6% and 22 vs 11% respectively).
Conclusion
This study identified hyponatraemia on discharge from hospital as a significant and potentially avoidable risk factor for readmission within 28 days.

DOI: 10.1530/endoabs.34.P51

Clinical practice/governance and case reports

P52

Clinical application of the levothyroxine absorption test

Muhammad Butt[1], Nidhi Gupta[1], Fiona Greenfield[1] & Najeeb Waheed[2]
[1]Peterborough City Hospital, Peterborough, UK; [2]Hereford County Hospital, Hereford, UK.

Background
Poor adherence with thyroxine treatment is a common problem faced by endocrinologists the world over. Abnormally elevated TSH levels, with rising levels of treatment is an ongoing management challenge, especially with patient insistence of compliance. There are a number of investigative tools advocated. We present a case managed through an observed thyroxine absorption test.
Methods
A 36-year-old woman was referred by her primary care practitioner, due to a persistent elevated TSH, leading to a prescribed dose of 450 μg of thyroxine daily. The patient insisted on good compliance with medication and after excluding malabsorption conditions, we arranged an observed thyroxine absorption test. We arranged for the patient to present to the hospital daily for 5 days; be watched taking the prescribed dose and her mouth checked to ensure swallowing and the patient then was not allowed the visit the bathroom for an hour after swallowing.
Results
The patient consented 2 weeks prior to the investigation. At the beginning of the 5-day her free hormone levels increased significantly. At the beginning of the 5-day, bloods later demonstrated FT_4 60.9, FT_3 14.4 and TSH 0.03, confirming she had now started taking her tablets. She complied with the thyroxine absorption test and the repeat blood investigations at the end of the 5 days later demonstrated FT_4 $>$100, FT_3 21.4 and TSH 0.03, thus confirming compliance with treatment and over-treatment with thyroxine.
Conclusions
The investigation demonstrated an over-prescription of thyroxine due to poor-compliance with treatment. We recommended the cessation of treatment for 14-day then recommencement at a lower dose with repeat testing after 2 months to test compliance. For difficult to manage poor-compliance cases, an observed thyroxine absorption test can be invaluable in identifying inadequate treatment due to poor compliance.

DOI: 10.1530/endoabs.34.P52

P53

Recurrent hypomagnesaemia, tackling modifiable risk factors

Moayed Alhelfi & Yared N Demssie
Royal Blackburn Hospital, Blackburn, UK.

A 68-year-old lady presented to the medical assessment unit with history of muscle cramps involving both hands and legs of 5 days duration. She also gave a history of diarrhoea of 2 weeks duration. Her background medical history includes hypertension, hypercholesterolemia and dyspepsia for which she was taking Irbesartan/hydrochlorothiazide combination tablets, simvastatin and omeprazole.
General physical examination revealed positive Chovstek's and Trausseau's signs. Her investigation results confirmed low adjusted serum calcium level of 1.22 mmol/l (normal range 2.15–2.6 mmol/l) along with low serum magnesium level of 0.14 mmol/l. Her serum 25 hydroxy-vitamin D level was also on the lower side at 14 ng/ml and PTH level was marginally raised at 8.3 pmol/l. ECG showed normal sinus rhythm with borderline QT duration at 450 ms.
She was treated with 10 mls 10% calcium gluconate in 100 mls 5% dextrose infusion over 5 min followed by 100 mls 10% calcium gluconate in 900 mls 5% dextrose infusion over 24 h. She also received 16 mmol of magnesium sulphate infusion in 1 l 5% dextrose over 24 h. Her symptoms resolved with this treatment and both her serum calcium and magnesium levels returned to normal at 2.28 and 0.75 mol/l respectively. However, she continued to have diarrhoea with an average frequency of 3-4 times per day. Her stool culture result was reported negative for bacterial pathogens but further investigation with flexible sigmoidoscopy confirmed the diagnosis of colitis for which she was started on a course of oral steroid therapy.
Despite on-going diarrhoea, the patient was discharged from hospital without any oral calcium or magnesium supplement. She also continued to take both irbesartan/hydrochlorothiazide and omeprazole tablets which further increased her risk for recurrent hypomagnesaemia. Not surprisingly the patient was readmitted 2 weeks later with recurrence of neuromuscular symptoms along with profoundly low serum calcium and magnesium levels of 1.4 and 0.15 mmol/l respectively. She was once again treated with both calcium and magnesium infusion until her serum levels returned to normal. She was also maintained on oral magnesium glycerol phosphate at a dose of 24 mmol/ day in three divided doses. Her hydrochlorothiazide tablet was stopped to reduce renal magnesium wasting and her omeprazole tablet was replaced with ranitidine. She has remained asymptomatic since and both her serum calcium and magnesium levels have stayed normal.
Conclusion
This case highlights the importance of identifying and correcting modifiable risk factors for recurrent hypomagnesaemia such as drugs as well as the need for prescribing maintenance oral magnesium supplementation in patients with ongoing magnesium loss.

DOI: 10.1530/endoabs.34.P53

P54

Thyrotoxicosis after severe hypothyroidism in a patient with a history of Hodgkin's lymphoma and neck irradiation

Krzysztof Lewandowski[1], Katarzyna Dabrowska[1], Jacek Makarewicz[2] & Andrzej Lewinski[1]
[1]Department of Endocrinology and Metabolic Diseases, 'Polish' Mother Memorial Research Institute, The Medical University of Lodz, Lodz, Poland; [2]Department of Nuclear Medicine and Oncological Endocrinology, Zgierz District Hospital, Zgierz, Poland.

Background
Neck irradiadion is associated with several complications including both hypo- and hyperthyroidism. We present a case of severe hypothyroidism, followed 5 years later by thyrotoxicosis.
Case description
A 27-year-old female presented with clinical and biochemical thyrotoxicosis (TSH 0.01 μIU/ml (ref. range: 0.27–4.2 μIU/ml); $FreeT_4$ 1.58 ng/dl (ref. 0.98–1.63 ng/dl); $FreeT_3$ 4.56 pg/ml (ref. 2.6–4.4 pg/ml). Clinical examination revealed tachycardia about 100 beats/min. and no obvious goitre. Autoimmune profile was suggestive of Graves' disease (anti-TSH-receptor antibodies (aTSHR) 16.69 IU/l (ref. 0–1.75), anti-thyroid peroxidase antibodies (aTPO) 1780 IU/ml (ref.: 0–34 IU/ml). She had a history of Hodgkin's lymphoma, diagnosed and treated with chemo- and radiotherapy (including the neck) at the age of 18. At the age of 20 she developed severe hypothyroidism (TSH $>$100 μIU/ml), with high titres of both aTPO (150 IU/ml) and aTSHR (37.56 IU/ml) antibodies. Thyroid function tests normalised after treatment with thyroxine (100 μg od). At the age of 26 she became 'anxious' and experienced 'heart palpitations'. She was found to have suppressed TSH, that remained suppressed even when the dose of thyroxine was reduced and then discontinued. After further 4 months she was found to have raised free T_3 (see above). Thyroid scintigraphy revealed a normal and homogenous iodine uptake (41%). The patient responded very well to treatment with low dose thiamazole (10 mg od).
Conclusions
Our case illustrates that after neck irradiation, even severe hypothyroidism can be followed by thyrotoxicosis. There is a possibility that in this case there was a gradual switch from a TSH receptor blocking antibodies into TSH receptor stimulating antibodies.

DOI: 10.1530/endoabs.34.P54

P55

Large testicular adrenal rest tumours in a patient with congenital adrenal hyperplasia: a consequence of poor drug compliance

Jeyanthy Rajkanna & So Oyibo
Peterborough and Stamford Hospitals NHS Foundation Trust, Peterborough, UK.

Introduction
Testicular adrenal rest tumours (TART) are benign corticotrophin-dependent tumours that occur in males with congenital adrenal hyperplasia (CAH). We present a patient with bilateral large TART as a consequence of poor compliance to treatment and follow-up for his CAH.
Case
A 25-year-old gentleman presented to the endocrine clinic in 2009 with a history of tiredness, reduced libido and bilateral large testicles, which he wanted surgically removed. He had been diagnosed with CAH during the antenatal period and commenced on steroid replacement therapy soon after birth. However, he stopped taking steroid replacement in 2000 because of domestic/family issues and although his testicles were felt to be lumpy in 2002, further follow-up and investigation was difficult. On examination he was well with normal secondary sexual features, but his testicles felt hard and three times the normal size.
Investigations
His biochemical test results are shown in the Table 1. An ultrasound confirmed large testicles. He was commenced on steroid replacement therapy and referred for orchidectomy and replacement prosthesis. Both testicles measured 8.5×5× 4 cm in size with no recognisable testicular parenchyma (stage 5): the histology was characteristic of TART. His serum testosterone levels fell to the lower limit of the normal range post-surgery.
Conclusion
Although not malignant, TART can result in irreversible damage of testicular tissue and infertility. Treatment with glucocorticoid replacement therapy can stabilise or repress growth of these tumours. This case highlights the importance of compliance to treatment and follow-up to prevent testicular damage as a result of TART in patients with CAH.

DOI: 10.1530/endoabs.34.P55

Table 1

Biochemical test	Results	Normal reference range
17-OH-progesterone	>152	<13 nmol/l
Testosterone	50.4	10–38 nmol/l
ACTH	139	0–50 nq/l
Dehydroepiandrosterone	12	1.6–11 nmol/l
FSH	<1	1–14 U/l
LH	<1	1–9 U/l
Prostate specific antigen	0.27	<2.5 μg/l
30-min cortisol (post-Synacthen)	354	>550 nmol/l
Sodium	144	133–146 mmol/l
Potassium	4.2	3.5–5.3 mmol/l

P56

A case of precocious puberty in a 4-year-old boy: differential diagnosis and treatment goals

Ella-Grace Kirton
University of Nottingham, Nottingham, UK.

Introduction
Precocious puberty is uncommon in males and presents an interesting differential that can be narrowed down by an understanding of endocrine physiology. This case illustrates this and also led the author to explore the research basis for the treatment goals in this condition.
Case
A boy of 4½ years was referred to paediatric endocrinology clinic due to concerns about tall stature and pubic hair growth noted over the last 2 months. His past medical history was unremarkable although his father reported longer recovery times from minor illness than the rest of the family. There was no significant family history and his parents were average height and underwent puberty at average ages. He was above the 99.6th percentile for height and on the 98th for weight. Examination was unremarkable besides the fact that he was at Tanner stages 2 and 3 for genital development and two for pubic hair. Testes were prepubertal. Blood results revealed a low FSH (<0.5 IU/l), low cortisol (73 μmol/l), raised androstenodione (4.4 nmol/l) and his bone age was ten. These, alongside clinical presentation and urine steroid profile, confirmed the diagnosis of simple virilising congenital adrenal hyperplasia.
Discussion
Precocious puberty is defined as the appearance of signs of puberty before the age nine in boys. Its estimated incidence is 1:5000–1:10 000 (both sexes) but is far rarer in males. It presents an interesting differential and requires swift diagnosis to prevent adverse effects on the child's growth, development and future physical and mental health. Assumptions are made about the importance of 'normal' stature to quality of life, however evidence for this is lacking in the literature. Likewise, many beliefs on the impact of precocious puberty on male children are based on studies on female patients. The author has concluded that there may be further scope for research in these areas.

DOI: 10.1530/endoabs.34.P56

P57

Fixed dose radioactive iodine treatment for hyperthyroidism: experience of a district general hospital

Rashmi Manjunatha, Katherine Harris, Sarfraz Nawaz, Rajni Mahto & Peter Horrocks
Department of Diabetes and Endocrinology, Warwick Hospital, Warwick, UK.

Background
The short- and long-term clinical outcome of patients receiving radioactive iodine treatment (RAI) differs in various studies. There is little consensus regarding the most appropriate dose of RAI to be administered. The range of activities currently prescribed varies between 200 and 800 MBq, with majority of patients receiving 400–600 MBq. In our centre, all patients receive a standard fixed dose of 400 MBq.
Aim
To assess the cure rate with fixed dose (400 MBq) RAI therapy.
Patients and methods
This is a retrospective study of all patients treated with RAI at this hospital over a 3-year period. All patients received a fixed 400 MBq dose. TSH and FT_4 levels were recorded at diagnosis, the time of RAI, 6 months and 1-year post-RAI. Anti-thyroid medication was discontinued for 10 days prior to RAI therapy. Failure rate in terms of persistence of hyperthyroidism at the end of 6 months and 1 year was calculated.
Results
Mean age was 59 years (range: 25–88 years). Of the total 59 patients, 16 (27.12%) were male and 43 (72.88%) were female, 19 (32.2%) had Graves' disease, 19 (32.2%) had multi-nodular disease and in 21 (35.5%) patients, thyrotoxicosis was of unknown aetiology. At the time of diagnosis, 37% (22 patients) had goitre, 14% (eight patients) had thyroid ophthalmopathy, 22% (13 patients) were positive for antiTPO antibody and 94.91% (56 patients) were pre-treated with anti-thyroid drugs. 48 patients (81.36%) were either hypothyroid (28 patients, 47.45%) or euthyroid (20 patients, 33.89%) at 6 months. 11 patients (18.6%) were hyperthyroid at 6 months. At 12 months, 45 patients (76.27%) were either hypothyroid (12 patients, 20.3%) or euthyroid (33 patients, 55.9%) and 14 patients (23.7%) had persistence of hyperthyroidism.
Conclusion
In our centre, a standard 400 MBq dose of RAI has a cure rate of 81.36% at 6 months and 76.27% at 12 months, which is in line with the figures quoted in the literature.

DOI: 10.1530/endoabs.34.P57

P58

Investigation of inpatient hyponatraemia in a teaching hospital

Ploutarchos Tzoulis, Devaki Nair, Rebecca Leyland, Emma Woolman, Nick Martin & Pierre Marc Bouloux
Royal Free Hospital, London, UK.

Introduction
Hyponatraemia is the most common electrolyte disorder in hospitalised patients and is associated with significant morbidity and mortality.
Methods
This retrospective study included all inpatients with serum sodium (sNa) ≤128 mmol/l at any point during hospitalisation at a teaching hospital over a 3-month period (1st March 2013 to 31st May 2013). Demographic, clinical and laboratory data obtained from patients' case notes and laboratory information system were reviewed with the aim to study the investigation of hyponatraemic inpatients.

Results
139 patients (69 males and 70 females) with a mean age (±S.D.) of 70.2±16.1 years were identified over this 3-month period. The median length of hospital stay was 12 days and the inpatient mortality rate was 17.3%.
61.9% of patients had their volume status assessed. The proportion of patients having appropriate laboratory investigations was: 38.1% for serum osmolality, 37.4% for urine osmolality, 35.2% for urine sodium concentration, 61.1% for thyroid function tests, 31.6% for serum cortisol. Only 28.8% of patients had paired serum and urine osmolality and sodium measured.
Patients with sNa ≤125 mmol/l ($n=87$) were four times more likely to have paired serum and urine osmolality and sodium checked and six times more likely to have serum cortisol measured than patients with sNa 126–128 mmol/l ($n=52$).
Patients under the care of medical specialities were more likely to have the appropriate tests than patients under surgical specialities.
Only 20 patients (14.4%) were referred to endocrine services. 80% of these patients had complete clinical and laboratory assessment compared to 5% of patients not referred to endocrine services. The median time interval between onset of hyponatraemia and referral was 4 days and referred patients had on average two endocrine consultations.
Discussion
Hyponatraemia is frequently underinvestigated. Endocrine specialist input can facilitate the appropriate investigation of hyponatraemia.

DOI: 10.1530/endoabs.34.P58

P59

Unexpected adrenocortical carcinoma 8 years after diagnosis of adrenal incidentaloma

Mahender Yadagiri & David Barton
Shrewsbury and Telford NHS Foundation Trust, Telford, UK.

Adrenal incidentalomas (AI) are incidentally discovered adrenal masses on imaging studies requested for reasons unrelated to adrenal pathology with a prevalence of 8% in autopsy and 4% in radiologic series. Although most AI are non-functioning benign adenomas, their increasing prevalence presents diagnostic and therapeutic challenges. We report a case of adrenocortical carcinoma (ACC) in an asymptomatic 83-year-old man, 8 years after being diagnosed with AI. ACC is an uncommon malignancy carrying a high mortality.
In accordance with current guidelines, the AI was followed up with sequential CT at intervals of 6, 12 and 24 months which measured 2.0 cm throughout. Biochemical testing revealed only incomplete cortisol suppression with low dose dexamethsaone. Some 8 years later, he had a staging CT in view of a non-healing tongue ulcer which showed a prominent right adrenal mass measuring up to 9.0 cm.
The radiological characteristics were suggestive of ACC and in addition a low attenuation lesion was identified in the liver. Repeat biochemistry showed persistent subclinical hypercortisolism and normal plasma catecholamines. Right laparoscopic adrenalectomy was performed with uncomplicated postoperative recovery. Six months later, he presented with haemoptysis and CT abdomen showed progressive liver metastases requiring chemotherapy (Mitotane). We have reviewed the guidelines from the literature including AAES and AACE (2009), ACR (2012), NIH (2002) which limit follow-up recommendations of small AI to 5 years.
The initial size of adrenal lesion would have hardly warranted surgical intervention on size criteria alone. This patient had subclinical cushings syndrome (SCS) but data from randomised trials are lacking to guide the optimal management of SCS. A postulated strategy is to consider adrenalectomy for younger patients (<40 years) and those with disorders that are potentially attributable to autonomous glucocorticoid secretion (recent onset or worsening of underlying hypertension, diabetes mellitus, obesity or osteoporosis).

DOI: 10.1530/endoabs.34.P59

P60

Characteristics, comorbidities and aetiology of hospitalised patients with hyponatraemia

Ploutarchos Tzoulis & Pierre Marc Bouloux
Royal Free Hospital, London, UK.

Introduction
Hyponatraemia is the most common electrolyte abnormality encountered in hospitalised patients.
Methods
This retrospective study included all inpatients with serum sodium ≤128 mmol/l at any point during their hospital stay between 1st March 2013 and 31st May 2013. Full review of medical case notes and laboratory results was undertaken in order to determine the comorbidities, drug history and aetiology of inpatients with hyponatraemia.
Results
139 patients (69 males and 70 females) with a mean age (±S.D.) of 70.2±16.1 years were identified over this 3-month period. Most patients (77.0%) were under the care of medical specialities, with the specialities most frequently represented being geriatrics (16.5%), hepatology (14.4%), general medicine (13.7%), oncology (8.6%), cardiology (5.8%), neurology (5%) and renal (5%).
The most common comorbidities were hypertension (50.4%), lung disease (28.8%), diabetes (25.2%), arrhythmia (25.2%), liver disease (25.2%), active malignancy (24.5%), heart failure (21.6%), and chronic kidney disease (21.6%).
The most frequently prescribed drugs were proton pump inhibitors (49.6%), opioids (28.8%), loop diuretics (27.3%), ACE-inhibitors (26.6%), K-sparing diuretics (13.7%), thyroxine (12.2%).
Only 41.7% of patients had the aetiology of hyponatraemia recorded in the medical notes. Among these patients, the largest proportion (46.6%) had hypovolaemic hyponatraemia (24.1% due to diuretics, 13.9% due to gastrointestinal Na losses, 8.6% due to poor oral intake). 34.5% had euvolaemic hyponatraemia, in particular 10.3% had SIADH due to pneumonia, 8.7% SIADH due to miscellaneous causes, 8.6% SIADH of unknown cause and 6.9% malignant SIADH. Finally, 18.9% had hypervolaemic hyponatraemia, either due to cirrhosis (10.3%) or due to heart failure (8.6%).
Discussion
Most inpatients with hyponatraemia had multiple comorbidities and did not have their aetiology of hyponatraemia recorded. Taking into account their variety of aetiologies and the wide distribution into various specialities, different models of care delivery incorporating expert input may be necessary to improve hyponatraemic patients' care.

DOI: 10.1530/endoabs.34.P60

P61

Endocrine complications of thalassemia and its treatment: a local experience

Serena Deller[1], Radhika Mistry[1], Wyn Burbridge[1], Shivan Pancham[2], Christine Wright[2] & Parijat De[1]
[1]Department of Diabetes and Endocrinology, City Hospital, Birmingham, UK; [2]Sickle Cell and Thalassemia Centre, City Hospital, Birmingham, UK.

Introduction
Treatment of β thalassemia major results in excessive iron deposition in most tissues including the endocrine glands.
Aim
To find out the prevalence of endocrine complications in our thalassemia-endocrine clinic as set out by the UK thalassemia society guidelines.
Methods
Clinic notes and electronic results of all patients attending Birmingham City Hospital for the 2-monthly thalassemia-endocrine MDT clinic (2010–2012).
Results
Of 21 patients (mean age 29 years, 11 M:10 F, 19 South Asian, one Chinese, and one Cypriot), 20 were transfusion dependant and two post allogenic BMT. 4/19 (21%) were currently non-compliant with chelation (19/21 patients).
Multiple endocrinopathies were common: three (14%) had one endocrinopathy, nine (42%) had two, four (14%) had three and five (23%) had four or more endocrinopathies (older, mean age 35 years).
The commonest endocrinopathies were: hypogonadism (16/21–76%), hypoparathyroidism (9/21–43%), osteoporosis (9/21–43%), diabetes mellitus (8/21–38%) and hypothyroidism (8/21–38%). 9/21 (43%) had cardiac overload with LV systolic dysfunction.
Mean fructosamine in diabetics improved over time (377 in 2010 vs 336 in 2012).
Osteoporosis was associated with hypogonadism (8/9), hyperparathyriodism (7/9), diabetes (3/9), low vitamin D (9/9) and low calcium levels in (6/9) patients. 50% showed improvement in serial DEXA scanning (uptake only 50%) following treatment. Hypothyroidism (mostly asymptomatic) had poor relationship to serum ferritin or compliance with chelation. Delayed puberty and amenorrhoea were the commonest manifestations of hypogonadism.
Conclusion
High prevalence of endocrinological problems in thalassemia patients makes regular surveillance essential both for early detection and treatment of complications especially since thalassemics are living longer. Compliance with chelation and high DNA rates were particularly noted in those with hypogonadism (testosterone therapy) and diabetes (BM monitoring, retinopathy

screening and clinic attendance). Regular follow-up, on going education and multi-disciplinary support remains vital for improving prognosis in these patients.

DOI: 10.1530/endoabs.34.P61

P62

Juvenile granulosa cell tumour of the ovary presenting with hyperprolactinaemic amenorrhoea and galactorrhoea

Ahmed Iqbal[1,2], Alexandra Lubina-Solomon[1,2] & Jonathan Webster[1]
[1]Sheffield Teaching Hospitals, Sheffield, UK; [2]University of Sheffield, Sheffield, UK.

Background
Ovarian granulosa cell tumours are rare, aggressive, hormonally active neoplasms.
Case
A 16-year-old, nulliparous, Eastern European woman presented with a 9-month history of secondary amenorrhoea and a 2-month history of galactorrhoea. She denied headache or visual symptoms. Past medical history was unremarkable with menarche at age 14 and a previously normal menstrual cycle. She had never used hormonal contraception. Only medications were paracetamol and ibuprofen. On examination, visual fields were full and cranial nerves were intact. General examination was unremarkable. Pituitary bloods demonstrated suppressed gonadotrophins (LH <0.1 IU/l, FSH <0.1 U/l) and an elevated serum prolactin at 7081 mIU/l (ref range 102–496 mIU/l). A macroprolactin screen was negative. Serum oestradiol was significantly elevated at 7442 pmol/l and serum β HCG was undetectable. Thyroid function tests and a short synacthen test were satisfactory. MRI of the pituitary showed no focal abnormality. We considered a malignant ovarian source for the high serum oestradiol. Serum inhibin was elevated at 2734.7 ng/l. MRI of the pelvis showed a 4.8 cm mass within the right ovary, no evidence of metastatic disease and a normal uterus and left ovary. The patient underwent a right-sided salpingo-oophorectomy. Histology confirmed the mass to be a juvenile granulosa cell tumour (FIGO stage 1a). On immunohistochemical analysis, tumour cells did not express prolactin. The patient defaulted from follow-up after surgery.
Conclusion
We present a case of juvenile granulosa cell tumour producing oestradiol and inhibin. Based on immunohistochemistry, we postulate that observed hyperprolactinaemia and the subsequent clinical sequelae were caused by oestrogenic stimulation of pituitary lactotroph cells, a biochemical state analogous to pregnancy. Our case illustrates an uncommon but important cause of hyperprolactinaemia, which, if missed, could have significant adverse consequences for the patient.

DOI: 10.1530/endoabs.34.P62

P63

Analysis of hyponatraemic inpatients who died in a teaching hospital

Ploutarchos Tzoulis & Pierre Marc Bouloux
Royal Free Hospital, London, UK.

Introduction
Hyponatraemia is associated with increased inpatient mortality, but there is debate about whether hyponatraemia *per se* contributes to mortality or is merely an epiphenomenon of severe illness.
Methods
This retrospective review of medical records included all inpatients with serum sodium (sNa) ≤ 128 mmol/l who died at a teaching hospital over a 3-month period. The aim of this study was to examine the clinical course and the potential contribution of hyponatraemia to death.
Results
Among 139 hyponatraemic patients, 24 patients (17.3%), 13 males, 11 females, with a mean age ($\pm$S.D.) of 72.9 ($\pm$14.2) years died during hospitalisation. Nine patients had hypovolaemic hyponatraemia, eight had SIADH, three had hypervolaemic hyponatraemia, and four had hyponatraemia of unknown type. Fatal cases had a median length of hospitalisation of 21.5 days with a median exposure to sNa levels ≤ 128 mmol/l of 4 days. On admission, nine patients had sNa ≤ 128 mmol/l, seven had sNa 129–134 mmol/l, and eight were normonatraemic. The median sNa on admission was 132 mmol/l, at the lowest point during hospitalisation 124 mmol/l and at the time of death 135.5 mmol/l. At the time of death, seven patients (29.1%) had sNa ≤ 128 mmol/l, three (12.5%) had sNa 129–134 mmol/l, 11 (45.8%) were normonatraemic, and three (12.5%) were hypernatraemic. The primary causes of death were: pneumonia (six cases), malignancy (five cases), cirrhosis (four case), heart failure (three cases), visceral perforation (two cases), myocardial infarction (one case), acute kidney injury (one case), stroke (one case), and cerebral vasculitis (one case). The case notes review did not identify any cases of direct contribution of hyponatraemia to death.
Discussion
This study did not identify any cases where recognised complications of hyponatraemia contributed to death, but it cannot exclude a causal link through unknown physiological effects.

DOI: 10.1530/endoabs.34.P63

P64

Elevated hounsfield units and large tumour size on radiological imaging are both suggestive of functionality in incidental adrenal tumours

Dilip Abraham, Jadwiga Raasz, Edward Kearney & Stonny Joseph
East kent Hospitals University and NHS Foundation Trust, Kent, UK.

Widespread use of CT and MRI scan has led to the identification of incidental adrenal tumours. The need to determine functionality often results in a battery of investigations that are a drain to scarce resources and are frequently normal. Studies to identify tumour radiological features that suggest functionality and hence enable targeted investigations are few in the literature. To this effect we set out to analyse which features on imaging that are best predictive of functionality.
We retrospectively reviewed 17 patients who have been diagnosed with an incidental adrenal mass. The imaging features and endocrine investigations carried out on these patients were noted and data on patients with functional tumours (F) were compared with those with non-functional tumours (NF). Data are expressed as mean + S.D. and a P value of <0.05 (unpaired t-testing) deemed significant.
Patients had a mean age 68 years, eight males. Four patients with functional tumours were identified based on the results of comprehensive endocrine investigations; two phaeochromocytomas and two adrenocortical carcinomas. The tumour hounsfield units and size were higher in these patients with functional tumours when compared to non-functional ones although not significantly so; size (F vs NF, 68.25 + 45.39 vs 23.76 + 8.022 mm, $P=0.11$), hounsfield units (F vs NF, 64.25 + 10.34 vs 38.29 + 31.47, $P=0.13$). There was a weak but positive relationship between size and hounsfield units with a correlation coefficient of 0.32. 24-h urine catecholamine and free cortisol were more commonly abnormal in these functional tumours.
Our study will suggest that adrenal tumour size of above 30 mm and a hounsfield unit of above 40 are predictive of functionality and only these should be subjected to extensive endocrine investigations. The lack of statistical significance could be due to the small sample size of our study and will indicate the need for larger studies to confirm our findings.

DOI: 10.1530/endoabs.34.P64

P65

Spontaneous hypoglycaemia in a non-diabetic patient with insulin antibodies

Una Graham, Laura McQuillan & John Lindsay
Mater Hospital, Belfast, UK.

A 58-year-old non-diabetic Caucasian man was admitted with a capillary glucose of 1.9 mmol/l following an episode of confusion and disorientation. During his admission he had frequent episodes of nocturnal and early morning hypoglycaemia with capillary glucose <3.0 mmol/l. After 21 h of supervised fasting he was symptomatic with plasma glucose 2.3 mmol/l, insulin >1000 mU/l and C-peptide 19.6 µg/l. Sulphonylurea screen was negative. Given the magnitude of serum insulin, insulin antibodies were measured and were positive. Serum insulin was corrected for the presence of antibodies using PEG precipitation yet remained elevated.
CT imaging of pancreas was normal. Endoscopic ultrasound demonstrated a hyper-echoic abnormality in the tail of the pancreas measuring 13×11 mm. He subsequently attended for calcium stimulated venous sampling which demonstrated high insulin production throughout the gland with no localisation. The patient started carbohydrate supplementation and 5 mg daily prednisolone with resolution of hypoglycaemia over 8 weeks.
Insulin autoimmune hypoglycaemia is a rare condition characterised by extremely high levels of insulin in the presence of anti-insulin antibodies. It is the third leading cause of hypoglycemia in Japan but has rarely been described in the non-Asian population. Making the correct diagnosis is important to avoid an unnecessary pancreatic surgical procedure on a hypoglycemic patient.

DOI: 10.1530/endoabs.34.P65

P66

Severe hypercalcaemia requiring emergency haemodialysis due to *postpartum* hypophysitis and thyroiditis

Jing Mia Gao[1], Benjamin Whitelaw[1,2], Omar Mustafa[2] & Salim Cheeroth[1]
[1]Department of Acute Medicine, King's College Hospital, London, UK; [2]Department of Endocrinology, King's College Hospital, London, UK.

A 34-year-old African woman presented with a 4-month history of profound lethargy, weight loss and a 3-day history of vomiting, fever, confusion, and drowsiness. She had given birth to her third child 6 months previously. Her pregnancy and delivery were uncomplicated and she had breastfed for 5 months prior to discontinuing due to exhaustion.

Investigations showed severe hypercalcaemia (4.94 mmol/l) and undetectable PTH. Hypercalcaemia was unresponsive to intense fluid rehydration, calcitonin and i.v. pamidronate. Emergency haemodialysis was required to reduce serum calcium to a safe level.

Further investigations showed severe cortisol deficiency (34 nmol/l) with undetectable ACTH and mild T_3 thyrotoxicosis, (fT_3 7.5 pmol/l, normal fT_4 and suppressed TSH). Pituitary MRI showed no pituitary mass or significant radiological abnormality. Insulin stress test performed 2 months after discharge confirmed profound hypocortisolaemia (cortisol <30 nmol/l at all test points with normal growth hormone response (peak of 10.80 μg/l).

The patient was treated with hydrocortisone, initially i.v. and then converted to orally (10 mg twice a day). Her symptoms dramatically improved following cortisol replacement and normalisation of serum calcium.

The patient subsequently developed hypocalcaemia, with serum calcium falling to a nadir of 1.79 mmol/l. This was treated and corrected with alfacalcidol and calcium supplementation.

Discussion

This case demonstrates the emergency management of severe hypercalcaemia including the use of dialysis. The etiology of the hypercalcaemia is a combination of thyroiditis and cortisol deficiency, both of which are known to cause this. Cortisol deficiency is, in turn, due to ACTH deficiency in the presence of an apparently structurally normal pituitary gland. The presumed underlying cause is combined *postpartum* hypophysitis and *postpartum* thyroiditis. This combined presentation with hypercalcaemia is extremely rare.

The treatment given led to a subsequent hypocalcaemic phase which can be regarded as a form of hungry bone syndrome.

DOI: 10.1530/endoabs.34.P66

P67

Parathyroid adenoma presenting solely as acute necrotising pancreatitis in an adolescent girl

Mallikarjuna Jeeragi, Shabber Zaveri, Nagaraj Palankar & Karthik Prabhakar
Manipal Hospitals, Bangalore, Karnataka, India.

Parathyroid-adenoma is a rare cause of primary-hyperparathyroidism in children. We report a case where in a 15-year-old girl presented with acute necrotising pancreatitis as its sole manifestation.

This high-school girl presented with 2-month history of nausea, vomiting, upper abdominal pain, and weight-loss. An episode of severe acute abdomen led to her hospitalisation. At admission, she was tachycardic, dehydrated and had tenderness in peri-umbilical area.

Preliminary investigations: normal FBC, ESR, renal and liver function tests, urinalysis, lipid profile (including triglycerides), but serum corrected calcium was 3.95 mmol/l (2.1–2.65). Ultrasonography and then CT scan confirmed acute necrotising pancreatitis. Her serum calcium levels were 2.65 and 2.87 mmol/l on two separate occasions in the last 2 months. Hypercalcaemia was hence felt to be the underlying cause for acute pancreatitis. Subsequent investigations: parathyroid-hormone (PTH) 261 pg/ml (10–65), vitamin D 18.5 ng/ml (30–80), phosphorus 2.3 mg/dl (2.3–4.7), TSH 1.3 μIU/ml (0.7–5.5), prolactin 22 ng/ml (3–24); neck ultrasound showed parathyroid adenoma.

Pancreatitis was managed conservatively. Despite aggressive i.v.-rehydration, calcium level remained high; she was started on salmon calcitonin s.c. injections, initially at 4 mg/kg body-weight 6th-hourly; then increased to 8 mg/kg dose. After 2 days, she also needed i.v. pamidronate 60 mg to reduce hypercalcaemia. When calcium was 2.95 mmol/l, she underwent parathyroid-adenomectomy. Consequently, PTH reduced to 7.8 pg/ml. Histopathology showed well-encapsulated parathyroid-adenoma. Post-operatively, despite pre-operative i.m. injection of vitamin D 600 000 units, she developed hungry-bone syndrome with symptomatic hypocalcaemia 1.87 mmol/l, and hypomagnesaemia 0.8 mg/dl (1.6–2.6); she received i.v. calcium and magnesium for 5 days, and then oral supplements plus 1-α-calcidol, with good response.

Literature mentions association of hypercalcaemia with pancreatitis but is uncommon especially in adolescents. Underlying pathophysiology in hypercalcaemia-induced-pancreatitis is unknown. Suggested mechanisms from animal-studies include hypercalcemia-provoked-pancreatic injury via secretory block, accumulation of secretory proteins, and possibly activation of proteases. Since serum calcium levels can acutely increase in parathyroid-adenoma patients, calcium levels should be closely monitored in such situations.

DOI: 10.1530/endoabs.34.P67

P68

Pseudo-secondary hyperparathyroidism due to vitamin D deficiency and coexisting familial hypocalciuric hypercalcaemia

Anna Louise Mitchell[1], Richard Bliss[2] & Simon H S Pearce[1]
[1]Department of Endocrinology, Royal Victoria Infirmary, Newcastle upon Tyne, UK; [2]Department of Endocrine Surgery, Royal Victoria Infirmary, Newcastle upon Tyne, UK.

A 54-year-old lady presented with malaise, weakness, and constipation. She was found to be mildly hypercalcaemia (adjusted calcium 2.68–2.76 mmol/l; reference range 2.12–2.60 mmol/l) and was referred to local endocrinology services. Her PTH was 79 ng/l (range 10–60) and a diagnosis of primary hyperparathyroidism was made. Imaging to localise a parathyroid source was negative. She was referred to the endocrine surgeons at the Royal Victoria Infirmary, Newcastle, for consideration for parathyroidectomy and was subsequently listed routinely for neck exploration. However, her serum TSH was noted to be elevated (7.04 mU/l) and she mentioned that she could not tolerate levothyroxine tablets prescribed for primary hypothyroidism. To centralise her care, she was referred to endocrinology at the RVI regarding this. When she was seen, she complained of limb pain and had a proximal myopathy. Her vitamin D level was <10 nmol/l and the possibility of secondary hyperparathyroidism was discussed. High dose vitamin D was prescribed, in addition to levothyroxine in syrup form, and a recommendation to postpone surgery was made.

The patient felt that she was being denied a curative operation and sought a second, and then third, opinion from other endocrine surgeons. These agreed that her vitamin D deficiency should be addressed prior to contemplating surgery. When her vitamin D levels were replete, her symptoms had improved but not entirely resolved. Her adjusted serum calcium was 2.63 mmol/l, with a magnesium level at the upper end of the reference range (0.97 mmol/l; range 0.70–1.00 mmol/l) and a modestly elevated PTH (64 ng/l). A calcium:creatinine clearance ratio calculated from a 24 h urine calcium estimation was 0.007. She underwent DNA sequencing for the six exons of the calcium sensing receptor (CASR) gene and was found to be heterozygous for a pathogenic duplication in exon 4 (741 dupT), predicting a frameshift.

Common problems, in this case vitamin D deficiency, may coexist with much rarer conditions such as familial hypocalciuric hypercalcaemia. Careful investigation prevented the patient from undergoing an unnecessary surgical procedure.

DOI: 10.1530/endoabs.34.P68

P69

An unusual cause and an unusual complication of Cushing's syndrome

Zana Ameen, Rakhi Kakad, John Watkinson & Kristien Boelaert
University Hospital Birmingham NHS Foundation Trust, Birmingham, UK.

We present a 71-year-old gentleman who was referred with a 3-month history of tiredness and proximal myopathy, diarrhoea, rapid weight loss and new onset diabetes mellitus.

On examination, he had features consistent with cortisol excess including thin skin, abdominal striae, proximal muscle wasting and peripheral oedema to his thighs. Neck examination revealed a palpable 2-cm right-sided thyroid nodule.

Laboratory investigations showed hyperglycaemia and hypokalaemia. Endocrine testing confirmed Cushing's syndrome caused by ectopic ACTH secretion (ACTH 264.5, NR 0–46) with the remainder of his pituitary function and pituitary MRI within normal limits. Fine-needle aspiration of the thyroid nodule was consistent with medullary thyroid carcinoma (MTC) and serum calcitonin was 4568 (NR <4.8).

He was commenced on metyrapone to reduce cortisol secretion. CT scanning demonstrated multiple lymph node metastases in his chest and normal adrenal glands. His inpatient stay was complicated by episodes of breathlessness and hypoxia. CTPA showed ground glass changes and atelectasis. Broncho-alveolar

lavage revealed *Pneumocystis jirovecii* and treatment with co-trimoxazole was initiated. Over the course of 3 weeks his condition stabilised and he was fit for surgery. He tested negative for HIV.
He underwent total thyroidectomy with radical neck dissection and his cortisol and ACTH levels improved postoperatively. Histology confirmed MTC as the source of ectopic ACTH.
This case illustrates both an unusual cause of Cushing's syndrome and an unusual complication from immunosuppression associated with cortisol excess. Medullary thyroid cancer can secrete ACTH in up to 40% of cases, but rarely causes an ectopic ACTH syndrome. Some degree of immunosupression is a recognised complication of cortisol excess but *P. jirovecii* infection has been described rarely.

DOI: 10.1530/endoabs.34.P69

P70

Not every Gestational Diabetes is Mellitus!

Zana Ameen
Heart of England NHS Foundation Trust, Birmingham, UK.

A 21-year-old lady referred for an endocrine review from labour ward HDU after developing polyurea and polydipsia following a difficult labour which was complicated by severe post-partum haemorrhage.
She was passing about 500 ml of urine per hour and drinking around ten jugs of water a day. Her bedside observations were stable and her capillary blood glucose level was 4.7 mmol/l. She was referred to ITU where she had a stat dose of IV-desmopressin. Further history revealed 2 weeks history of polyurea and polydipsia prior to labour.
Laboratory investigations showed a plasma glucose of 4.9 mmol/l. Her urea and creatinine were normal and her eGFR was >90 ml/min. Her sodium level was high at 146 mmol/l. Her serum osmolality was 296 mosm (275–295) and her urine osmolality was 78 mosm (300–1 000). Her urinary sodium excretion was 24 mmol.
Further tests revealed normal TSH of 2.63 mIU/l and a normal cortisol of 557 nmol/l. Her prolactin level was 2 630 IU/l and she continued to lactate normally. Her pituitary MRI scan was unremarkable, so diagnosis of Sheehan's syndrome was excluded.
She was started on oral desmopressin for suspected diabetes insipidus and she responded well; with urine output going down to 2 420 ml. Unfortunately, she did not stay in hospital to have her fluid deprivation test done.
This was an unusual case of gestational diabetes insipidus which is frequently under-diagnosed because polyurea is often considered normal during pregnancy. It is thought to be due to excessive placental vasopressinase in the 3rd trimester which breaks down ADH. It is very important to diagnose and treat gestational diabetes insipidus early because it can lead to significant morbidity and mortality (rapid onset hypernatraemia leading to central pontine demyelination and baby's death).

DOI: 10.1530/endoabs.34.P70

P71

A rare endocrine cause of severe resistant hypoglycaemia

Zana Ameen
Heart of England NHS Foundation Trust, Birmingham, UK.

A 68-year-old lady presented to hospital with several weeks' history of paroxysmal symptoms including sweating, pre-syncope and syncope. She also had 2 months history of weight loss. She was a chronic heavy smoker, but did not drink any alcohol and did not take any regular medications.
On examination, she appeared cachectic and had a non-tender palpable liver. There were no signs of decompensated chronic liver disease. Her bedside capillary blood glucose was 0.9 mmol/l (NR 3.8–6.1).
Laboratory investigations showed hepatitic picture of deranged LFTs (ALP 383, ALT 50, GGT 635, and Bilirubin 15). Abdominal ultrasound and staging CT scans confirmed heterogenous solid masses in right lobe suggestive of liver metastasis.
Further tests revealed a high AFP level of $>1\,000$ μg/l (NR <5.8). Her hepatitis screen and auto-immune profile were normal. Her lab glucose was 1.2 mmol/l. Her insulin, C-peptide and IGF1 concentrations were undetectable, but her serum IGF2 level was high at 102 nmol/l (NR 0–10). Her liver biopsy confirmed high-grade hepatocellular carcinoma.
Unfortunately, her hypoglycaemia was resistant to continuous IV-dextrose infusion and she continued to have frequent hypoglycaemic episodes with seizures and brain damage. She became too unwell and deemed unfit for surgical intervention or any other treatment for debulking the tumour size. She gradually deteriorated and eventually passed away.
This case illustrates a rare cause of hypoglycaemia as a paraneoplastic phenomenon due to high IGF2 secretion by the tumour cells. Treating hypoglycaemia in such cases can be challenging as they tend to be severe and resistant to glucose replacement. Several other treatment options have been tried in some case reports, but nothing proven to be effective.

DOI: 10.1530/endoabs.34.P71

P72

Enigma of an 18 year Acromegalic Window: what your mind knows but eyes may still miss?

Danielle Foucault & Parijat De
Deparment of Diabetes and Endocrinology, City Hospital, Birmingham, UK.

A 46-year-old Asian male attended our endocrine unit in April 2013 following a referral from his GP for the investigation of a neck mass. Serum TSH level was normal and USS revealed a euthyroid multinodular goiter. He mainly complained of increasingly frequent and severe headaches, polydypsia, polyuria, perspiration, and night sweats.
On examination, the patient had prognathism, an enlarged and protruding tongue, thickened lips, prominent supraorbital ridges and a multi-nodular goiter. There was no hand tremor or sweating. He had spade-like hands preventing him from wearing his wedding ring. He was 6 feet 4 inches tall.
Random IGF1 was elevated (103 nm/l) and OGTT showed non-suppressible GH secretion (15.06 mIU/l at 120 min). MRI revealed a mildly enlarged pituitary gland. He has been referred for surgery. Having finally diagnosed him with Acromegaly, some of the preventable but now irreversible tissue changes and long-term complications nevertheless remain.
Medical diagnosis of Acromegaly, a classic endocrine condition with slow progression, is often done at first glance from clinical features. There is an estimated 7–12 years time-lag between first symptom and disease diagnosis. Despite our patient coming into contact with a whole host of medical and surgical specialities (since symptom onset over 18 years ago), he remained undiagnosed. Potential confounding factors in this delay could have been his ethnicity and gradual evolvement of clinical features over the years.
This case highlights that even with classical clinical features of acromegaly, there may be quite a wide ranging 'Acromegalic Window' between first presentation and clinical suspicion to eventual diagnosis and the potential for reduction in the length of this window. Furthermore, our patient's extensive past medical history and numerous medical encounters highlights the importance of patient assessment from a holistic view-point taking into account previous medical history, including a keen eye for evolution of patterns in symptoms and signs.

DOI: 10.1530/endoabs.34.P72

P73

Adrenal masses-a bleeding problem

You-Jin Chang, Angela Alina Khan, Sobia Arshad & Ian Scobie
Medway Maritime Hospital, Gillingham, UK.

Adrenal haemorrhage is comparatively rare. A 64-year-old female presented with acute right sided abdominal and chest pain shortly after a left knee replacement. She had recently started treatment dose low molecular weight heparin for presumed DVT for persistent pain in the left knee. A subsequent Doppler study of left leg was negative for DVT but an abdominal US revealed a solid lesion at the upper pole of the left kidney. CT urography showed large bilateral adrenal masses. No clinical or biochemical evidence of adrenal endocrine excess or deficiency was demonstrated. She became persistently pyrexial during admission, but no source of sepsis was found. At endocrine review, repeat CT scan with adrenal washout protocol demonstrated bilateral adrenal masses that had markedly reduced in size suggesting resolving bilateral adrenal haemorrhage. A 0900h plasma cortisol level, urinary normetadrenaline and metadrenaline excretion and a plasma aldosterone:renin ratio were all normal. Adrenal imaging review was consistent with bilateral adrenal haemorrhage (BAH) with no evidence of underlying adrenal tumour.
The incidence of BAH is estimated to be ~4.7–6.2 cases/million. Nontraumatic adrenal gland haemorrhage is rare. Causes include idiopathic, stress, haemorrhagic coagulopathy, neonatal stress, or underlying adrenal tumours. The perioperative period after orthopaedic surgery is associated with an increased risk of

a haemorrhagic event. From documented case reports, adrenal haemorrhages after orthopaedic procedures commonly present with abdominal pain and pyrexia. CT is the recommended choice of modality to diagnose adrenal haemorrhage. When adrenal insufficiency complicates BAH immediate treatment with replacement corticosteroids is mandatory.
This case demonstrates a rare case of post-operative BAH. It underlines the importance of recognising adrenal haemorrhage in the differential diagnosis of unexplained abdominal pain and pyrexia since a missed diagnosis could be fatal.

DOI: 10.1530/endoabs.34.P73

P74

Abstract Withdrawn.

DOI: 10.1530/endoabs.34.P74

P75

Stuttering priapism

Ahmed Imran Siddiqi
University College London, London, UK.

Introduction
Here, I present an interesting case of stuttering priapism in a 48 years old man. He was 30 years old when he was diagnosed with this condition. He is being treated with cyproterone acetate with significant improvement in his symptoms.
Clinical case
A 30 years old man (now 48) presented to A&E with painful prolonged erection (priapism). He denied any history of trauma, blood disorders, use of recreational drugs, over the counter drugs or similar symptoms in the past. He had not noticed any change in his libido and was married for previous 6 years. Testosterone was 18.2 (7.8–31 nmol/l). He received the standard treatment by urology team with blood drainage from penis and pseudo-ephedrine injection. Detumescence was achieved with this treatment. His penile doppler confirmed high flow priaprism with flow reaching 60 cm/s. He presented a few more times to A&E with similar complaint and doppler confirmed high flow priapism. He was commenced on cyproterone acetate for control of his stuttering priapism. His symptoms of priapism were well controlled with cyproterone acetate 50 mg four times a week earlier on during his treatment but now 25 mg three times a week is sufficient. His testosterone remains in the low normal range or slightly below the normal range (latest 5.6 nmol/l) with no systemic symptoms. His DEXA scan showed *T* score at hip 1.3 and at spine 2.0. He continues on cyproterone acetate with good control of his symptoms.
Conclusion
We present here the presentation and management of a patient with stuttering priapism. Different treatments have been tried with variable response. In the absence of randomised controlled trials the best treatment option still remains under discussion for such patients.

DOI: 10.1530/endoabs.34.P75

P76

Recovery of adrenal function in confirmed Addison's disease

Mark Baxter, Sondra Gorick & Francesca Swords
Norfolk and Norwich University Hospital, Norwich, Norfolk, UK.

Background
Addison's disease is characterised by immune mediated destruction of the adrenal glands. This process is widely deemed to be irreversible. We present a case of confirmed Addison's disease where adrenal function appears to have spontaneously recovered.
Case report
A 37-year-old presented in 1997 with classical Addison's disease: pigmentation, dizziness, weight loss, and nausea. There was no relevant family history. Random cortisol was 43 and 56 nmol/l at 30 min post synacthen. Adrenal antibodies, ACTH and renin were not checked. Treatment with hydrocortisone 20 mg waking and 10 mg mid-afternoon, and fludrocortisone 50 μg daily led to an excellent recovery.
On subsequent registration with a new endocrinologist, gradual hydrocortisone dose reduction was suggested. Pre- and post-dose cortisols were also checked, and the pre-dose level found to be surprisingly high: 359 nmol/l. The hydrocortisone dose was therefore reduced further. Repeat Synacthen testing provoked a rise in cortisol to 596 nmol/l, and so hydrocortisone was cautiously withdrawn, with no impact on symptoms, electrolytes or blood pressure. Off treatment, 0900 h ACTH was initially elevated at 86 ng/l but has fallen to 13 ng/l, Synacthen testing remains normal, and the patient remains well after 6 months off treatment, 16 years after his initial diagnosis. Renin remains elevated so fludrocortisone has been continued. Adrenal antibody tests are now positive and aldosterone levels are pending.
Discussion
This is the third and most clear cut reported case of spontaneous recovery from Addison's. However, we have no models to predict which patients are more likely to recover than others, and so vigilance in the follow-up and monitoring of Addison's is vital. Finally, this case demonstrates the importance of patient involvement in the management of long term conditions, and has absolutely relied on detailed educational input from a specialist nurse to maintain his safety.

DOI: 10.1530/endoabs.34.P76

P77

Hypercalcaemia: a mixed family picture

Leanne Hunt[1], Robert Robinson[1], Pallia Shillo[1], Treena Cranston[2] & Rajesh Thakker[3]
[1]Chesterfield Royal NHS Hospital Trust, Chesterfield, Derbyshire, UK; [2]Oxford Genetic Laboratories, The Churchill Hospital, Oxford, UK; [3]Academic Endocrine Unit, Oxford University, Oxford, UK.

Hereditary hypercalcaemia can be a diagnostic challenge. We present a family with autosomal dominant hypercalcaemia that illustrates this. A 48-year-old man was referred with asymptomatic long standing hypercalcaemia (2.8–3.04 mmol/l), with plasma PTH levels between 45 and 48 ng/l (normal 15–65), and a high urinary calcium excretion, (24 h calcium collection 10.4 mmol/l (2.5–7.5), with high calcium excretion indexes) all consistent with primary hyperparathyroidism. His mother had been referred to a different hospital with hypercalcaemia, and biochemistry consistent with primary hyperparathyroidism. Isotope scan had confirmed a parathyroid adenoma, and calcium levels normalised after parathyroidectomy from >3 to 2.56 mmol/l, with histology confirming an adenoma. Parathyroid uptake scan in our male patient was normal. Screening of his son and daughter confirmed both having hypercalcaemia (2.9–3.0 mmol/l), PTH levels 30–45 ng/l, and urinary calcium excretion indexes 12–18 (<22 consistent with familial hypocalciuric hypercalcaemia FHH).
The father had genetic testing which confirmed a C to T nucleotide substitution at codon 15 of *AP2S1* (adaptor-related protein complex two, sigma one subunit), c.43C$>$T. This results in replacement of the amino acid arginine with cysteine (p.Arg15Cys) and confirmed FHH type three.
FHH has autosomal dominant trait with high penetrance, and is characterized by elevated serum calcium and is usually asymptomatic. Three types have been characterised. Type one and type two have mutations in the *CASR* and *GNA11* gene respectively, type three FHH is due to a mutation in *AP2S1*.
It is important to note that father and children had differing phenotypes with father having a high and children a low calcium excretion index. This case highlights the importance of taking a detailed family history, along with biochemical assessments and genetic analyses of close family in patients with hypercalcaemia.

DOI: 10.1530/endoabs.34.P77

P78

Severe hypercalcaemia and acute kidney injury secondary to Graves' thyrotoxicosis

Thinzar Min, Lawrance Cozma & Silas Benjamin
Princess of Wales Hospital, Bridgend, UK.

A 46-year-old woman presented with 2 months history of thirst, polydipsia, polyuria, constipation, three-stone weight loss, and abdominal pain. She had no other significant medical problem except from personality disorder, which was controlled with antipsychotic medications. She denied use of over-counter

medications. She had been off lithium for 5 years.
On examination, she was dehydrated, tachycardic and appeared anxious. She had palmar erythema, fine tremor, firm symmetrical non-tender goiter, and no evidence of thyroid ophthalmopathy.
Investigations revealed acute kidney injury (serum urea of 13.5 mmol/l and serum creatinine of 140 mcmol/l), severe hypercalcaemia (corrected serum calcium of 3.15 mmol/l), normal serum phosphate level (PO_4 1.45 mmol/l), magnesium of 0.73 mmol/l, and suppressed PTH of 0.6 pmol/l. Subsequent thyroid function test showed suppressed TSH of <0.03 mU/l, FT_4 of 68 pmol/l and FT_3 of 27.8 pmol/l. She scored 50 points on Burch-Wartofsky score, which is suggestive of thyroid storm. Carbimazole, propranolol and i.v. glucocorticoid were commenced.
All investigations for causes of hypercalcaemia were proved to be normal. Her serum calcium level gradually declined and renal function returned to normal once thyroid function improved. At 8 weeks follow-up, TFT continued to improve and serum calcium level remained within normal range. TRAb was elevated. We concluded that hypercalcaemia was due to Graves' thyrotoxicosis.

DOI: 10.1530/endoabs.34.P78

P79

Patient feedback on receiving copies of clinic correspondence

Ram Prakash Narayanan, Preeti Chiran, Steven J McNulty, Upendram Srinivas Shankar & Niall Furlong
Department of Diabetes and Endocrinology, St Helens and Knowsley NHS Trust, St Helens, UK.

Background and aim
The Department of Health and NHS Plan both recognise that sending patients copies of clinic correspondence can help inform and empower them. At St Helens and Knowsley Teaching Hospitals NHS Trust, Endocrinology Clinic we routinely provide patients with a copy of the clinic correspondence sent to their General Practitioner (GP), unless patients specifically opt out. Our aim was to evaluate patients' views on receiving such copy correspondence following outpatient endocrinology review.
Methods
150 patients who had visited the endocrinology clinic were posted a questionnaire to explore their views about receiving a copy of the letter sent to their GP. Patients were asked to rate their degree of agreement, or disagreement, with nine statements regarding the advantages, or disadvantages, of receiving copy correspondence.
Results
68 patients responded (45%). Most (94.1%) agreed that receiving a copy of the clinic letter helped them understand more about their condition and 97.1% felt more informed and involved in their care and treatment; 98.5% reported that receiving the letter helped them understand what had been conveyed to their GP regarding them, and 97.1% indicated it allowed them to check the content for accuracy. 95.6% stated it reassured them that their GP had also received the specialist's letter, informing them when to contact their primary care team regarding any recommended treatment changes. Very few patients (7.4%) reported anxiety and distress on receiving the copy letter. Approximately 10% of patients needed to show the letter to someone else to help them understand it. Overwhelmingly, 100% of respondents stated that routinely receiving copies of correspondence was a good idea.
Conclusions
This study indicates that our endocrine patients like receiving copy correspondence of their clinic letter and reinforces existing government policy recommending this practice.

DOI: 10.1530/endoabs.34.P79

P80

Carbimazole induced agranulocytosis: a case report in the setting of a young female, on low dose and duration of treatment of more than one year

Hiang Leng Tan & Isy Douek
Musgrove Park Hospital, Taunton, UK.

Introduction
The incidence of agranulocytosis with thionamide therapy is uncommon, ranging between 0.2 and 0.5 percent. Most reports document agranulocytosis after short duration of high dose Carbimazole in older patients. We report a 32-year-old lady with Graves' disease who developed neutropaenic sepsis secondary to Carbimazole therapy on 5 mg daily and on treatment for the last 13 months.
Case report
Our patient was diagnosed with Graves's thyrotoxicosis when she was 14 weeks pregnant. She was commenced on high dose Carbimazole then reduced to 5 mg on alternate days. She had an uneventful delivery of a healthy baby boy. *Post-partum*, her dose of Carbimazole was increased to 5 mg daily.
Four days before she was admitted, she had developed fever, sore throat and an itchy rash. A blood test done by her GP at day 4 showed a neutrophil count of 0.75 and she was urgently admitted. She was treated as neutropaenic sepsis and her Carbimazole was stopped. She was clinically euthyroid on admission (TSH <0.01, T_4 13.7, and T_3 5.8). A joint decision was made by the ENT and Endocrinology team for her to have an urgent total thyroidectomy. A dermatology opinion was sought and confirmed the widespread confluent rash to be related to carbimazole therapy. Her neutrophil count recovered to 2.04 at day 5 and she had her total thyroidectomy 5 days later.
Discussion
All patients should receive written and verbal warnings to look out for the typical warnings signs of possible agranulocytosis, not only prior to starting anti thyroid drugs but also at each clinic visits if possible. Our case serves as a reminder that agranulocytosis with Carbimazole can happen even at low dose with duration of treatment of more than 12 months in a young age group.

DOI: 10.1530/endoabs.34.P80

P81

The patient with broken heart

Alexandra Lubina Solomon[1,2], Ahmed Iqbal[1,2], Barney Harrison[2], Jonathan Webster[2] & William Bennet[2]
[1]The University of Sheffield, Sheffield, UK; [2]Sheffield Teaching Hospitals, Sheffield, UK.

A 58-year-old gentlemen presented with severe neckache, sweating, feeling hot, and cold. PMH included hypertension and MI. On examination, he was clammy, with blood pressure (BP) 205/107 mmHg. Laboratory results showed leucocytosis 28.5 and TroponinT 2002 ng/l (reference range <14.0). ECG showed inferolateral ST depression. CT angiogram showed a normal aorta and a 6 cm right adrenal mass. An echocardiogram showed severe LV impairment and normal valves.
Phenoxybenzamine, nitrates, diuretics and digoxin were commenced. After 2 days, the patient developed fever (38.5 °C), acute kidney injury, hyponatraemia and a DIC-like picture. Intravenous fluids and antibiotics were given for suspected sepsis. Capnocytophaga canimorsus rods were later grown from blood cultures and the patient recollected that he had recently been bitten by a dog. Following treatment, the patient made a full recovery with subsequent normalisation of BP. Urinary collections for adrenaline, noradrenaline and metanephrine showed values of 40 763, 30 378, and 142.6, respectively (upper reference ranges 490, 93 nmol/24 h and 1.7 μmol/24 h). Coronary angiography confirmed two vessel disease. Surprisingly, TTE performed about a week after admission showed normalisation of LV function. The patient underwent laparoscopic right adrenalectomy and is currently well.
Background
TakoTsubo cardiomyopathy (broken heart) is a condition characterised by transient systolic dysfunction. It appears to relate to contraction abnormalities secondary to increased levels of stress hormones. Clinical and ECG presentations resemble ACS.
Capnocytophaga canimorsus are anaerobic, gram-negative rods present in the oral flora of canines. Only about 200 cases of human infection have been reported worldwide. In humans, Capnocytophaga can cause fatal sepsis and DIC with increased mortality rate.
In summary, we describe a unique case of the patient with phaeochromocytoma crisis accompanied by transient severe LV dysfunction and features of ACS, which could be a part of TakoTsubo cardiomyopathy, who developed life-threatening Capnocytophaga canimorsus sepsis, following a dog bite.

DOI: 10.1530/endoabs.34.P81

P82

Do you know where the hypo box on the ward is? A revealing audit…

Michelle Willmott
The North West London Hospitals NHS Trust, London, UK.

Episodes of in-hospital hypoglycaemia are uncommon but serious events, which cost the NHS financially and cost the patient in terms of poor clinical outcomes, and rarely, mortality. The National Patient Safety Agency reported that in 2007, one in four adult diabetics experienced an episode of hypoglycaemia whilst in hospital. Moreover, one in 30 required i.v. glucose or i.m. glucagon therapy, and between 2003 and 2009 there were four deaths involving insulin overdose[2].

Part of the best practice in managing in-hospital hypoglycaemia is the use of hypo boxes, which are brightly coloured and easily recognisable for emergencies. The Joint British Diabetes Society produced guidelines on the hospital management of hypoglycaemia in adults in 2010, in which they recommend that all hospital trusts use hypo boxes. However, this audit has shown that doctors' knowledge of where to find these hypo boxes is poor (initial audit results showed that only two out of 20 doctors (10%) knew where it was). Lack of knowledge of the hypo box location self-negates their existence, and poses a significant patient safety issue. Nonetheless, this audit has also shown that this deficit is easily corrected using a simple intervention like a teaching session (re-audit showed an improvement in knowledge from 10 to 95%).

On a more fundamental basis, this audit has demonstrated that new interventions, such as the introduction of a hypo box, are not easily welcomed into the clinical environment. In order for clinicians to change their current practice, reinforcement and encouragement are needed; otherwise these important interventions are at risk of being, quite simply, overlooked.

DOI: 10.1530/endoabs.34.P82

P83

Plasma 25-hydroxycholecalciferol before and after supplementation in paediatric oncology patients from Scotland: a time-series cross-sectional study

Raquel Revuelta Iniesta[1], Ilenia Paciarotti[1], Jane McKenzie[1], Mark Brougham[2] & David Wilson[3]
[1]Queen Margaret University, Edinburgh, UK; [2]Royal Hospital for Sick Children, Edinburgh, UK; [3]University of Edinburgh, Edinburgh, UK.

Background
25-hydroxycholecalciferol (25(OH)D) deficiency is prevalent in the Scottish paediatric population. Paediatric oncology patients are at even higher risk of becoming deficient because they spend more time indoor, often have an inadequate dietary intake and increased 25(OH)D catabolism during treatment. We aimed to assess 25(OH)D and parathyroid hormone (PTH) levels before and after supplementation in Scottish children with cancer.
Methods
Plasma 25(OH)D and PTH were measured twice in patients aged <18 years, diagnosed and treated for cancer. Supplementation was prescribed according to UK guidelines, and consisted of macronutrient (enteral, $+/-$ parenteral nutrition) and micronutrient (vitamin D, multivitamins, $+/-$ macronutrient). 25(OH)D and PTH ranges were classified according to the Scottish laboratory reference; 25(OH)D: suboptimal (50–75 nmol/l), insufficient (25–50 nmol/l) and deficient (<25 nmol/l) and PTH: 1.6–7.5 pmol/l. Descriptive statistics, Spearman's and Wilcoxon's test were performed.
Results
40 patients had plasma 25(OH)D and PTH available before and after supplementation. Median (IQR) time between diagnosis and supplementation was 0.29 (0.2–0.7) years. At baseline, plasma 25(OH)D was suboptimal in 23% of patients and below suboptimal in 62.5%. 7.5% had hyperparathyroidism and 12.5% hypoparathyroidism. 17.5% received macronutrient supplementation. Of these 43% remained with plasma 25(OH)D below suboptimal and 7% with high PTH. Median (IQR) 25(OH)D decreased from 77 (42–81) to 54 (19–89) nmol/l and PTH increased from 3.6 (1.45–5.75) to 4.3 (1.45–7.15) pmol/l. Conversely, those supplemented with micronutrients (82.5%) had a significant improvement in 25(OH)D ($P<0.001$; r -0.7); median (IQR) increased from 26.5 (15–37) to 65.5 (44–87) nmol/l; yet 20% remained below suboptimal levels. Median (IQR) PTH decreased from 3.3 (2.3–5) to 3 (2–4.4) pmol/l ($P>0.5$). 25(OH)D and PTH did not significantly correlate ($P>0.05$).
Conclusion
25(OH)D deficiency was highly prevalent, only improving following micronutrient supplementation. To optimise the 25(OH)D status, regular monitoring alongside appropriate supplementation, which must be adapted to the specific needs of this population, should be incorporated into clinical practice.

DOI: 10.1530/endoabs.34.P83

P84

Long-term follow-up of patients treated with tolvaptan for resistant hyponatraemia

Malik Asif Humayun, Rosina Elliot, Michael Cummings, Partha Kar, Darryl Meeking & Iain Cranston
Queen Alexandra Hospital, Portsmouth, UK.

Background
We previously published data for short-term outcomes in patients with SIADH-related hyponatraemia unresponsive to fluid restriction and treated with tolvaptan. In light of concerns over longer-term side-effects we have revisited the topic and extended both group size and follow-up period to determine longer-term safety and treatment implications.
Aims/methods
We report ongoing treatment outcomes (case-note review) for 25 patients (age 71 ± 5 years, 60% females) consecutively treated with tolvaptan as an in-patient for confirmed SIADH with persistent Na <125 mmol/l despite removal of reversible causes and 48 h fluid restriction, and include longer-term outcome data (re-treatment/readmissions/mortality) for 2 years follow-up.
Results
Concordance with locally agreed criteria for tolvaptan use remains high:
Short-term outcomes remain good, 92% achieve target of treatment (Na >125 mmol/l with clinical improvement) after a mean of 3.4 ± 2.6 days treatment, 8% partial response (rise >5 mmol/l but not achieving 125 with initial therapy). No patient experienced a Na rise >12 mEq/24 h, hypernatraemia, drug-associated liver injury or CNS-myelinosis.
Longer-term outcomes were less good, mortality rates in this population was 40% within 3 months and 56% over the follow-up period. Underlying causes for SIADH were found to be malignancy-related in 60% (of whom 90% were undiagnosed at presentation). Mortality in those with malignancy was 46% at 3 months and 75% over the follow-up period. 24% of patients required re-treatment for recurrent hyponatraemia after tolvaptan discontinuation (100% of whom had relapsed within a week and had underlying malignancy).
Conclusions
Tolvaptan use remains safe and effective in-patient short-term therapy option in the group we have identified and these data merit investigation for its wider use. Sodium level 1 week after discontinuation is a good indicator of re-treatment/longer-term therapy needs, nearly all of whom have malignancy. Thus, the criteria we have set locally to indicate tolvaptan use also identifies a group who should receive urgent investigation for underlying malignancy.

DOI: 10.1530/endoabs.34.P84

P85

A rare cause of primary adrenal insufficiency

Bethany Watt, Catrin Barwick, Catrin Searell & Tony Wilton
Betsi Cadwaladr University Health Board (West), Bangor, UK.

Primary adrenal failure is a rare disorder with a prevalence in developed countries of 93–140 per million and an incidence of 4.7–6.2 per million. Autoimmune adrenalitis is the main cause (80%) followed by tuberculosis (15%). The remaining 5% of cases represent rare disorders with bilateral adrenal haemorrhagic infarction secondary to primary antiphopholipid syndrome being very rare (0.5%).

A 57-year-old female presented with a 3-week history of abdominal pain, nausea, vomiting and a fever. She had previously been diagnosed with primary antiphopholipid syndrome after presenting with transient ischaemic attacks and a cerebrovascular accident. Lupus anticoagulant and anticardiolipin antibodies of both IgG and IgM isotypes were positive. She was anticoagulated with warfarin. Examination found her to be unwell with pyrexia up to 38.9 °C, hypotension and tachycardia. A urinary tract infection was suspected and treated with antibiotics. Lack of clinical response lead to extensive differential diagnoses with none confirmed. Abdominal CT scan coincidentally revealed enlarged adrenal glands with evidence of haemorrhagic infarction. Investigations confirmed hypoadrenalism with 0900 h cortisol 80 nmol/l and ACTH 229.2 ng/l. Short Synacthen test: cortisol levels 0 min 85, 30 min 80 and 60 min 76 nmol/l. Hydrocortisone replacement resulted in rapid clinical improvement.

In most reported cases the diagnosis of haemorrhagic infarction of the adrenal glands in antiphopholipid syndrome was serendipitous as it was in this patient. The presentation of the case was typical of reported cases with fever (65%), abdominal pain (70%), hypotension (60%) and infection as the precipitating factor (40%). Whilst adrenal insufficiency is reported in only 0.4% of patients with antiphopholipid syndrome the typical symptoms and signs offer the potential for educated rather than chance diagnosis.

DOI: 10.1530/endoabs.34.P85

P86

A feminising adrenocortical carcinoma

Bethany Watt, Sarah Wenham, Kakali Mitra, Avril Wayte & Tony Wilton
Betsi Cadwaladr University Health Board (West), Bangor, UK.

Gynaecomastia is common, occurring in 30–50% of healthy men, and its aetiology is usually benign. Clinical features inclusive of recent onset, rapid progression, and loss of libido and muscle strength, indicate the need to exclude a malignant aetiology.

A 48-year-old man presented with an 18-month history of such clinical features. Investigations revealed cortisol 1136 nmol/l, 17-hydroxyprogesterone 6.6 nmol/l, androstenedione 13.3 nmol/l, DHEAS 21.8 μmol/l, urinary free cortisol $>$1380 nmol/24 h, testosterone 2.8 nmol/l, oestradiol 1261 pmol/l, FSH 0.1 U/l, LH 0.1 U/l, prolactin 598 μIU/ml, SHBG 51.3 nmol/l, fT_4 11.9 pmol/l, fT_3 2.9 pmol/l, TSH 1.6 mU/l, AFP $<$6 IU/ml and HCG $<$1 mIU/ml. Ultrasound of testes was normal but a CT scan demonstrated a 17$\times$13$\times$17.5 cm left adrenal mass, multiple liver metastases, extensive para-aortic lymphodenopathy and lung metastases. The impression formed was one of an oestradiol secreting adrenal carcinoma. At laparotomy a 1.6 kg left adrenal tumour was excised with as much nodal disease as could be safely removed. Histology confirmed adrenocortical carcinoma.

Adrenocortical carcinoma is a rare disease with an annual incidence of 1–2 per million. Feminising oestradiol secreting tumours account for $<$1% of cases. Peripheral conversion of high levels of androstenedione secreted by the tumour to oestradiol is thought to contribute to the high oestradiol levels. The ENSAT (European Network for the Study of Adrenal Tumours 2008) stage 4 (T4, N1, M1) disease this patient was found to have confers a very poor prognosis. Despite chemotherapy with mitotane, cisplatin, doxorubicin and etoposide, he died 8 months after presentation.

DOI: 10.1530/endoabs.34.P86

P87

Look before you leap… An adrenal mass and elevated metadrenalines may not be phaeochromocytoma

Iona Galloway, Vincent McAulay & Stewart Ferguson
Diabetes Day Centre, Crosshouse Hospital, Irvine Road, Kilmarnock, Ayrshire, UK.

A 73-year-old female with previous tuberculosis, primary hypothyroidism, depression and caecal cancer was referred to Endocrinology when interval CT scanning reported a 2.5 cm adrenal mass, stable in size over 4 years. No symptoms or signs of hormonal production were noted. Screening tests were abnormal: post-1 mg dexamethasone cortisol was 98 nmol/l, 24-h urinary free metadrenaline was elevated tenfold (3256 nmol/24 h, range 0–350) to a concentration reported to have high positive predictive value for phaeochomocytoma. Repeat sampling was confirmatory. Venlafaxine was suspected as causative and following discussion with psychiatry was substituted with sertraline. The 24-h urinary free metadrenaline normalised and has remained stable. Low-dose and high-dose dexamethasone suppression tests confirmed subclinical Cushing's syndrome related to an adrenal adenoma. Absent clinical features, reassuring DEXA and urinary cortisol:creatinine ratios favoured an observational approach.

Venlafaxine is a serotonin and noradrenaline reuptake inhibitor. Case reports have described modestly elevated catecholamine metabolites in patients prescribed venlafaxine with 24-h urinary noradrenaline elevated 1.6-fold[1] and plasma normetanephrine fourfold[2]. This case identified substantially elevated urinary metadrenalines at concentrations usually associated with phaeochromocytoma, which then normalised upon discontinuing venlafaxine. Caution is required when interpreting catecholamine results in patients prescribed SNRI's or other psychoactive medications due to the potential for false-positives[3] and elevated catecholamines metabolites do not necessarily indicate phaeochromocytoma. Reports in the endocrine literature which defined the sensitivity and positive predictive value of 24-h urinary catecholamine metabolites in diagnosing phaeochromocytoma[3] may require reappraisal in view of the increasing utilisation of SNRI's and other novel psychoactive medications known to interfere with catecholamine metabolism.

1. *Endocrine Abstracts* 2007 **14** P482.
2. *NEJM* 2011 **364** (23) 2268–70.
3. *JCEM* 2003 **88** 2656–66.

DOI: 10.1530/endoabs.34.P87

P88

Is demeclocycline safe and effective in the treatment of SIADH?

Shiva Mongolu & Azraai Nasruddin
Southampton General Hospital, Southampton, UK.

Background

Demeclocycline is a well-known treatment for syndrome of inappropriate ADH secretion (SIADH). We studied the use of demeclocycline for treatment of SIADH in our hospital to assess its efficacy and safety.

Materials and methods

A search was performed for all discharge summaries containing demeclocycline over period of 1 year. 40 results obtained of which five were excluded as demeclocycline was mentioned but not actually used. 35 admission episodes involving 27 unique patients were identified. 18 episodes excluded as they were on demeclocycline prior to admission. Data analysed for 17 episodes where demeclocycline was started in the current admission.

Results

Of the 17 episodes, prescription data was not available for two episodes and in one episode the use was transient ($<$5 days). The results showed that the mean rise in sodium level at day 5 was $>$3.1 mmol/l (data from seven patients) and the peak rise in sodium was at day 7 of $>$6 mmol/l (data from five patients). Two patients failed to show any significant response despite prolonged demeclocycline ($>$14 days). Three patients had to discontinue demeclocycline due to significant renal impairment.

Conclusions

Demeclocycline, though effective in the management of SIADH has a variable effect on sodium level, no significant change is observed in the first 72 h. In patients who respond, significant rise in Na is usually seen in 5–7 days. It is safe to initiate in an outpatient setting but serum Na should be monitored at 1, 2 and 4 weeks following initiation. It should be discontinued if significant renal impairment and/or hypernatremia develops.

DOI: 10.1530/endoabs.34.P88

P89

Low dose tolvpatan (7.5 mg) is effective in the management of SIADH in oncology patients (results from a retrospective audit at The Christie Hospital and Wythenshawe Pulmonary Oncology Unit)

Jennifer King[1,2], Angelos Kyriacou[1,2], Basil Issa[1,2], Paul Taylor[1,2] & Claire Higham[1,2]
[1]University Hospital of South Manchester NHS Foundation Trust, Manchester, UK; [2]The Christie Hospital NHS Foundation Trust, Manchester, UK.

Tolvaptan (a selective V2 receptor antagonist) is licensed for the inpatient management of SIADH induced hyponatraemia, a common complication in patients with malignancy. Licensed daily doses start at 15 mg but there is evidence that some patients have a rise in serum sodium (Na) of >12 mmol/l per 24 h in response to this. Lower initial doses (7.5 mg) may therefore be appropriate[1,2].

Methods

A retrospective case note audit was performed. Thirty-four oncology inpatients (mean (±S.D.) age 67±9 years, 15 males) were treated with tolvaptan (Nov 2009–Sept 2013). Twenty-six patients had small cell lung cancer (remaining diagnoses: renal cell carcinoma, oesophageal cancer, myeloma, testicular teratoma, cholangiocarcinoma and adrenal liposarcoma).

Results

Thirty-one patients had a trial of demeclocycline and fluid restriction prior to tolvaptan; 29 received endocrine team review. Nineteen met full biochemical criteria for SIADH (plasma osmolality <280 mOsm/kg, urine osmolality >100 mOsm/kg, urinary sodium >20 mmol/l). Three patients died. No incidents of central pontine myelinosis were recorded. Mean (±S.D.) (Na) pre-tolvaptan was 117±5 mmol/l. In the 28 patients receiving 7.5 mg, the initial increase (Na) /24 h was 7.4±5 mmol/l (27/28 showed a rise in (Na)); 4/28 patients had a rise in (Na)/24 h≥12 mmol/l. To achieve (Na) >130 mmol/l, three patients required escalation to a 15 mg dose. Twelve patients achieved a (Na) >130 mmol/l within 48 h with eight requiring tolvaptan on discharge. Mean (±S.D.) (Na) was 131±6 mmol/l on discharge. The six patients receiving an initial dose of 15 mg showed a mean (±S.D.) rise in (Na)/24 h of 13.8±4 mmol/l (5/6 were ≥12 mmol/l).

Conclusion

Tolvaptan 7.5 mg safely and effectively increased (Na) to >130 mmol/l in this group of patients. Higher doses of tolvaptan were more likely to cause a 24 h rise in (Na) outside of recommended limits. Further work is required to improve investigation and monitoring of hyponatremia in oncology patients.

DOI: 10.1530/endoabs.34.P89

P90

Teriparatide infusion for post-thyroidectomy hypocalcaemia: a case report

Khaliq Hamdan, Jawad Bashir, Ishrat Khan, Onyebuchi Okosieme, Neera Agarwal & Andrew Aldridge
Department of Endocrinology, Prince Charles Hospital, Merthyr Tydfil, UK.

Hypoparathyroidism post-thyroidectomy is a recognised complication and treated with calcium supplements, vitamin D, and rather recently with recombinant human parathyroid hormone therapy (teriparatide). We report a case of a 68-year-old female who developed refractory hypocalcaemia and hypomagnesaemia following thyroidectomy for a multinodular goitre. Her management was complicated by inflammatory bowel disease, surgically treated with an ileostomy *in situ*. Post-operatively she developed severe lethargy and paraesthesia, with hypocalcaemia (1.67 mmol/l) and hypomagnesaemia (0.53 mmol/l). She required multiple hospital admissions for i.v. replacement, as oral supplements were unable to maintain optimal levels. Henceforth, we resorted to trial of teriparatide injection, which was later switched to continuous infusion.

Teriparatide injection/infusion

She was commenced on teriparatide s.c. injections, once daily initially. However, despite increasing the dose to twice daily (40 μg BD), the optimal level of calcium was not sustained with consequent adverse symptoms requiring multiple hospital admissions for weekly i.v. calcium and magnesium infusions. Hence, she was started on a trial of continuous s.c. infusion of teriparatide. A stable and optimal level of calcium was achieved with 60 μg/day teriparatide with a significant reduction in the requirement for oral calcium and vitamin D supplementation, and no further need for i.v. therapy/hospitalisation to date (6 months).

Conclusion

Teriparatide has been shown to be effective in this lady with post-thyroidectomy hypocalcaemia with concomitant malabsorption, with no significant adverse effects to date. Her case illustrates an improved and sustained response to teriparatide infusion rather than s.c. injection, suggesting a physiological mechanism underlying the effectiveness of therapy. Although the treatment she received was relatively recent, she will require long-term monitoring for effectiveness, development of resistance, as well as other long-term side-effects. Further studies are still needed to evaluate the optimum means for management of hypocalcaemia in this challenging cohort.

DOI: 10.1530/endoabs.34.P90

P91

Kallmann syndrome, gender dysphoria, thrombophilia and multiple sclerosis: a complex case report

Aniruthan Renukanthan[1], Richard Quinton[2], Ben Turner[1] & Marta Korbonits[1]
[1]Barts and the London School of Medicine and Dentistry, London, UK; [2]University of Newcastle, Newcastle, UK.

A 34-year-old male with two decades of gender identity issues considered gender reassignment and attended pre-orchidectomy sperm-banking when he was found to be azoospermic. He described a history of difficulty learning to play the piano and of deep-vein thrombosis at 19 years. He was 187 cm tall with a span of 189.7 cm, and exhibited L-sided undescended testis, R testis 1.8×2.5×3.7 cm, bilateral prominent gynaecomastia, a female body habitus, micropenis, bimanual synkinesia and anosmia (confirmed with smell-testing). Low serum levels of testosterone (8 nmol/l) and gonadotropins confirmed hypogonadotrophic hypogonadism, consistent with Kallmann syndrome, though research-based genotyping showed no sequence changes in FGF8, FGFR1, KAL1, PROK2 and PROKR2 genes. Bone-density was impaired (*T*-score spine −1.9; hip −1.6) and MRI showed bilateral absence of olfactory nerves and bulbs. Low vitamin-D (21 nmol/l) prompted replacement. Thrombophilia screen identified low activated protein C resistance and a heterozygous Factor-V-Leiden mutation.

He has declined testosterone treatment pending assessment by a gender dysphoria clinic, though the possibility of future oestradiol treatment could be complicated by his previous DVT and Factor-V-Leiden status. Two years after his presentation he developed recurrent episodes of ipsilateral weakness and parasthesia in his limbs. Inflammatory spine lesions were identified which 2 years later were associated with white matter lesions in his brain in keeping with a diagnosis of multiple sclerosis (MS).

The question of validity of gender dysphoria diagnosis necessarily arises in the context of a prepubertal/apubertal individual, though psychosexual assessment was reassuring in this respect. Although male MS patients have been found to have reduced gonadotrophins, testosterone and sperm counts, suggesting an acquired effect of MS on hypothalamic regulation of the gonadotroph axis, in this case congenital hypogonadotropic hypogonadism is the likely diagnosis.

DOI: 10.1530/endoabs.34.P91

P92

An audit of clinical follow-up and management of adult patients with Turner's syndrome at the Royal Devon and Exeter

Elizabeth Whittle[1,2], Mollie Donohoe[1] & Antonia Brooke[1]
[1]Royal Devon and Exeter Hospital, Exeter, UK; [2]North Devon District Hospital, Barnstaple, UK.

Introduction

Turners' syndrome (TS) affects one in 2500 live births. Multiple comorbidities affect TS patients and recommendations for long-term management advocate annual review with a 'checklist' approach. This audit of current practice of a specialist TS clinic aims to identify gaps in patients' management.

Methods

A checklist of recommendations for health screening based on the National Turner's Syndrome Guidelines was developed. Data was collected from clinical encounters from June 2011 to Jan 2013 on a cohort of 37 patients seen in a specialist TS service.

Results

85% of the patients had had a cardiac MRI: 23% picked up cardiac abnormalities previously undetected on Echo. 89% had BP within target (21% on beta blocker):

30% had abnormal liver function tests. 92% under 50 years were receiving hormone replacement therapy (HRT); 88.2% oral. All with abnormal DXA scans received HRT. Fertility discussion had been documented in 36%.
Conclusions
This audit highlights the importance of a checklist approach to TS patients in a specialist service. The service will continue to ensure:
1) Routinely offering transdermal oestrogen preparations.
2) Protocol for initial and repeat cardiac MRI.
3) Early discussion re fertility and psychological and genetic support for this.
DOI: 10.1530/endoabs.34.P92

P93

Can a random cortisol predict outcome of short Synacthen test in non-acute patients with low pre-test probability?
Rajendran Bellan Kannan, Senthil Krishnasamy, Ananth Nayak, Biju Jose & Laks Varadhan
University Hospital of North Staffordshire NHS Trust, Stoke on Trent, UK.

Background
Short Synacthen test (SST) is being increasingly used in various clinical conditions, mainly for completion purpose of ruling out adrenocortical insufficiency rather than actively suspecting it (for instance hypothyroidism with tiredness, type 1 diabetes with hypoglycaemia). The aim of our retrospective analysis was to assess if a random cortisol could predict the outcome of SST.
Method
Data were collected on all SST done at the endocrine unit over the last 18 months. Pre-test probability was scored as low-risk or high-risk based on indication for the test, analysed from clinical letters. Baseline cortisol done as part of SST was taken as random cortisol; 30 min post 250 μg i.v. Synacthen injection >550 nmol/l was considered as normal response.
Result
Of the 346 patients who had SST over the study period, 233 (67%) were identified as low risk. 221 of these (95%) had a normal SST response. A baseline cortisol of 400 nmol/l was found to predict adequate cortisol response to SST in low risk patients, with following specificity: >200–66; >250–91; >300–91; >350–91 and >400–100%. 100 of the 233 patients (43%) had a baseline cortisol of >400 nmol/l. Random cortisol of <140 nmol/l could predict inadequate SST response.
Among high risk patients ($n=113$), 58% had a normal SST response. Interestingly, random cortisol of 400 nmol/l predicted adequate response in SST with following specificity: >200–87; >250–91; >300–91; >350–94 and >400–100%.
Conclusion
A random (baseline) cortisol of 400 nmol/l can be considered as a marker of adequate adrenocortical function in patients with low pre-test probability. SST could have been deferred on 43% of the low risk patients. Clinical triaging of cases referred for SST, based on pre-test probability, and a random cortisol could help reduce the number of SST performed in metabolic units. This approach would help prioritize clinical appointments for high risk patients and streamline limited resources.
DOI: 10.1530/endoabs.34.P93

P94

An unusual case of recurrent hypercalcaemia: sleeping parathyroid hyperplasia?
Una Graham[1], Fiona Eatock[2] & Brew Atkinson[1]
[1]Regional Centre for Endocrinology and Diabetes, Royal Victoria Hospital, Belfast, UK; [2]Endocrine Surgery, Royal Victoria Hospital, Belfast, UK.

A 60-year-old man presented with symptomatic primary hyperparathyroidism. At this time serum corrected calcium was 3.1 mmol/l (2.15–2.65 mmol/l) and after investigation bilateral parathyroid exploration was performed. A suspected adenoma was identified in the right inferior gland and removed. The remaining glands appeared normal. The adenoma was confirmed histologically. Early post-operative calcium was normal (2.48 mmol/l).
He remained well and calcium stayed within the normal range for 23 months. After this time serum corrected calcium started to rise to above the normal range with readings between 2.7 and 2.8 mmol/l. He remained asymptomatic. At 33 months calcium was 3.47 mmol/l with PTH of 231 pg/ml (15–70 pg/ml). A parathyroid subtraction scan did not identify an adenoma. A further bilateral neck exploration was performed. Intraoperatively what appeared to be a large left inferior parathyroid adenoma was identified and removed. Both superior parathyroid glands appeared normal. Histological examination of the removed tissue was in keeping with nodular/pseudoadenomatous hyperplasia rather than adenoma. Calcium settled post operatively to 2.39 mmol/l. MEN type 1 screening was negative.
Two possible explanations have been postulated for relatively early recurrence of hypercalcaemia after normalistaion of calcium by surgery. The first is temporary surgical damage which seems unlikely with the time course described here. The second is the rarely reported diverse functioning among multiple abnormal parathyroid glands whereby a large tumour may dominate as the main cause of hypercalcaemia and as a result suppress parathyroid secretion from another abnormal gland. We postulate that this occurred in the present case.
DOI: 10.1530/endoabs.34.P94

P95

Hypokalaemia attributed to liquorice use: not Allsorted!
Louise Hunter[1], Shailesh Agarwal[2], James Tymms[1] & Sushmita Pearce[1]
[1]Royal Albert Edward Infirmary, Wigan, UK; [2]University of Manchester, Manchester, UK.

We present two patients with significant hypokalaemia initially attributed to liquorice use.
Case 1
A 52-year-old engineer was noted to have a potassium level of 2.5 mmol/l (3.5–5.3 mmol/l) at a Well Man check-up. He had no significant past history, and took no medications. He was normotensive. The patient reported regular liquorice use. The hypokalaemia corrected with supplementation equivalent to 48 mmol K^+ daily, and with cessation of liquorice. Two months later, the hypokalaemia recurred (2.9 mmol/l). The patient reported that his mother also had low potassium levels. Further tests showed magnesium 0.66 mmol/l (0.70–1.00 mol/l) , bicarbonate 37 mmol/l (22–29 mmol/l), plasma renin activity 11.5 nmol/l per h (0.5–3.5 nmol/l per h) and urine potassium excretion 177 mmol/24 h (25–125 mmol/24 h). The patient was referred to Clinical Genetics and found to be a compound heterozygote for mutations in the SLC12A3 gene, in keeping with Gitelman's syndrome.
Case 2
A 72-year-old gentleman was referred to A&E with increased thirst and hypokalaemia of 2.8 mmol/l. He had suffered a cardiac arrest 5 months previously, when his potassium was 2.0 mmol/l. A defibrillator had since been implanted. His medications were amlodipine (for longstanding hypertension), atorvastatin, bisoprolol and warfarin. Hypernatraemia was noted, renin was undetectable, aldosterone within the normal range. The patient admitted to eating two bags daily of Pontefract cakes and Liquorice Allsorts. He agreed to stop. The hypokalaemia corrected with oral replacement, then spironolactone. Spironolactone was stopped once potassium reached the upper normal range. However, off spironolactone, potassium fell to 3.5 mmol/l. Renin remained undetectable, aldosterone increased above normal range. Imaging revealed a likely adrenal adenoma, suggesting Conn's syndrome.
Discussion
Whilst liquorice may cause of hypokalaemia, it may also exacerbate hypokalemia due to other causes, thus bringing them to attention. Renin and aldosterone levels were useful in these cases – hyperaldosteronism in Gitelman's is due to hyperreninaemia caused by volume contraction; liquorice results in pseudohyperaldosteronism with renin suppression.
DOI: 10.1530/endoabs.34.P95

P96

Pre clinic tests accelerate decision making, reduce delays in treatment and are highly popular with endocrinology patients and staff
Vidya Srinivas, Javier Gomez, Susan Kerry & Francesca Swords
Norfolk and Norwich University Hospital, Norfolk, UK.

Background
Thorough history taking and clinical examination is vital in the assessment of all medical patients. However, diagnosis of endocrine disease also relies on

appropriate biochemical tests. Previously in our unit, tests were only performed after new patients attended clinic. We proposed that diagnosis and management would be improved if this system was inverted.
Method
In 2009 clinicians, nurses and chemical pathology representatives reviewed available guidance and local practise to determine the key tests required to confirm or refute most common endocrine disorders. A pilot system of processing new referrals was then developed. The new system encouraged clinicians to categorise new referral letters into groups: for example immune thyroid disease, pituitary problem, possible PCOS. All patients identified to fall within a given group were then sent a standard letter requesting them to undergo the most important previously agreed tests prior to their hospital appointment. The secretarial team were then empowered to produce the letters and request the corresponding tests via a one click automated method. Long-term clinical outcomes have now been audited and patients and staff surveyed.
Results
The time from referral to confirming diagnosis and starting treatment (RTT) was halved: mean RTT fell from 11.8 to 5.7 weeks ($P<0.001$). Acute patients were also discharged from clinic earlier: mean 17–10.3 weeks ($P<0.005$). 94% patients surveyed had had their tests performed easily prior to attending their appointment. 100% of patients, 100% of hospital staff (consultants, secretaries, clinic and endocrine nurses), and 93% referring general practitioners felt the system improved patient care and should continue.
Summary
Performing investigations prior to new patient appointments is practical, popular with staff and patients, and has dramatically improved our referral to treatment statistics by reducing delays prior to diagnosis. This system could be used as a model for other departments.

DOI: 10.1530/endoabs.34.P96

P97

Carbimazole-induced antineutrophil cytoplasmic antibody positive vasculitis: a case report

Kah Fai Wong & Harit Buch
Wolverhampton Diabetes Centre, New Cross Hospital, Wolverhampton, UK.

Introduction
Vasculitis is a rare complication of anti-thyroid medications and is more common with propylthiouracil than carbimazole. Patients often have multisystem involvement although renal and dermatological manifestations are common. Although most cases resolve following withdrawal of the offending agent, not all do and rare instances of death have been reported in the literature. Immunosuppressive therapy can be used to improve organ function and prevent progression.
Case report
A 68-year-old man presented with fever, heat intolerance and weight loss. He was clinically and biochemically thyrotoxic with T_4 37.3 pmol/l and TSH <0.01 mU/l. There was no evidence of intercurrent infection. He was initiated on Carbimazole 40 mg once daily for presumed Graves' disease. Three weeks later he complained of diffuse erythematous skin rash affecting his trunk and limbs with typical features of vasculitic rash. Carbimazole was discontinued at the time. However, his clinical condition deteriorated with anorexia, weakness and weight loss. Investigation revealed worsening thyrotoxicosis and new onset renal failure. Urea 21 mmol/l, serum creatinine 194 ummol/l, ESR 73 ml/min, significantly raised myeloperoxidase ANCA antibody (36 IU/ml), strongly positive rheumatoid factor (227 IU/ml) and positive anti-nuclear antibody. Skin biopsy and renal biopsy were inconclusive and proteinase-3 ANCA antibody was negative. However, in view of the typical skin lesion, renal failure, raised ESR and high myeloperoxidase ANCA titre, diagnosis of carbimazole-induced vasculitis was made and he was commenced on intravenous methylprednisolone 500 mg for 3 days followed by oral corticosteroids. Radioiodine therapy (800 MBq) was administered along with beta blockers under close supervision.
He is now hypothyroid and is on thyroxine replacement. Despite high dose prednisolone he remains in renal failure, although his renal function has stabilised, skin lesions have subsided and repeat ANCA titre is negative.
In summary, we have presented a patient with carbimazole-induced vasculitis with partial response to withdrawal of offending agent and use of corticosteroids.

DOI: 10.1530/endoabs.34.P97

P98

Outcomes following pituitary surgery: a single centre audit

Konstantinos Manolopoulos[1,2], Andrew S Bates[2], Amjad Shad[3] & Assad Rahim[2]
[1]University of Birmingham, Birmingham, UK; [2]Heart of England NHS Foundation Trust, Birmingham, UK; [3]University Hospital Coventry, Coventry, UK.

Introduction
Pituitary surgery is associated with post-operative complications including hypopituitarism and visual field defects. In order to ensure that our surgical outcomes were comparable with other centres, we audited the outcome of a cohort of patients referred for surgery at a single centre.
Methods
A retrospective audit of 27 consecutive patients diagnosed with pituitary adenoma at Heartlands Hospital Birmingham, who underwent trans-sphenoidal surgery pituitary. Surgery was performed by a single neurosurgeon at our primary referral centre (University Hospital Coventry). Clinical notes and electronic records were used for auditing clinical outcomes. Between-group comparisons were made with *T*-test and ANOVA.
Results
Twenty-seven patients (12 males, 15 females; median age 61 years, age range 25–82 years) underwent surgery during the audit period. Main diagnoses were non-functioning pituitary adenoma (63%), acromegaly (22%) and Cushing's disease (4%); 85% ($n=23$) had a macroadenoma. Before surgery, 21 patients (78%) did not have any hormonal deficiencies, 18 patients (67%) had normal visual fields. Post-operatively, ten patients (37%) developed a new anterior hormonal deficiency (one axis affected, two patients; two axes, two patients; three axes, four patients); four patients (15%) developed new diabetes insipidus. In the macroadenoma group, 48% maintained full pituitary function post-operatively. Only one patient, operated on a recurrence of a previous non-functioning macroadenoma, developed a new visual field defect. No statistical differences in outcomes between micro- and macroadenoma groups.
Conclusion
Expectedly, most patients undergoing trans-sphenoidal surgery had a pituitary macroadenoma. While surgery is associated with the development of pituitary hormone deficiencies and new visual field defects, our data show that a large proportion of our patients maintained a normal pituitary function and did not develop new visual field defects. Our data are comparable to other trans-sphenoidal surgery centres in the UK and emphasise the importance of a single, dedicated pituitary surgeon.

DOI: 10.1530/endoabs.34.P98

P99

Hyperparathyroidism in early pregnancy: a case report

Yong Yong Ling, Agnieszka Falinska, David Woods, Tannaz Vakilgilani, Raj Tanday & Jeannie Todd
Imperial Centre of Endocrinology, Imperial College Healthcare NHS Trust, Hammersmith Hospital, London, UK.

A 36-year-old lady (gravida 2 para 0) presented with subfertility, dysmenorrhoea and mild hyperprolactinaemia (prolactin of 881 mU/l no macroprolactin). She was also found to be hypercalcaemic whilst on calcium supplements. Despite a negative home pregnancy test, her β-HCG was elevated at 1471 IU/l confirming she was pregnant.
She remained hypercalcaemic despite stopping calcium supplements and starting colecalciferol. At 9 weeks into her pregnancy, her corrected calcium (cCa) 2.67 mmol/l, phosphate 1.12 mmol/l, vitamin D 52.3 nmol/l and PTH 8.8 pmol/l. Urinary calcium creatinine clearance ratio of 0.0272 which was consistent with primary hyperparathyroidism (PHPT). MEN-1 genetic testing was negative. Her early foetal scans were normal.
She has a long history of liquorice ingestion hence was advised to stop in view of her hypercalcaemia. Her cCa ranged between 2.58 and 2.78 mmol/l. There were no nephrocalcinosis. Ultrasound (US) of parathyroid suggested a possible left inferior lesion in the tip of the thyroid measuring 8×3 mm which on US guided fine needle aspiration confirmed it was thyroid tissue (Thy 2). It was thought that further imaging would carry more risks than benefits.
She was referred to Endocrine surgeons and underwent a neck exploration with parathyroidectomy in her second trimester. Four parathyroid glands were identified and left inferior gland appeared to be an adenoma which was excised. Histology confirmed a 1.5 cm left inferior parathyroid adenoma weighing 2.27 g. Her biochemistry results (cCa 2.29 mmol/l, PTH 2 pmol/l)

post-parathyroidectomy confirmed she has been successfully treated. She went on to deliver a healthy girl at term.
Primary hyperparathyroidism in pregnancy is an important diagnosis to manage promptly due to the high risk of complications to both mother and foetus. It can be difficult to diagnosis due to the non-specific symptoms and maternal physiological changes. The definitive management option would be parathyroidectomy, usually in second trimester.

DOI: 10.1530/endoabs.34.P99

P100

Distinguishing between primary hyperparathyroidism and familial hypocalciuric hypercalcaemia: the role of genetic testing in patient with equivocal results

Yong Yong Ling, Agnieszka Falinska, Tannaz Vakilgilani, Raj Tanday & Jeannie Todd
Imperial Centre of Endocrinology, Imperial College Healthcare NHS Trust, Hammersmith Hospital, London, UK.

A 64-year-old gentleman was referred with persistent hypercalcaemia following two previous parathyroidectomies from an external hospital. He was found to be hypercalcaemic incidentally by his GP in 2011. Prior to surgery in January 2011, his corrected calcium (cCa) was 2.83 mmol/l, PTH 1.9 pmol/l, vitamin D 38 nmol/l, 24 h urine calcium creatinine clearance ratio (24 h UCCR) was 0.0135. Histology from his 1st neck exploration revealed one hyperplastic parathyroid gland. A further parathyroid gland (hyperplastic), two lymph nodes and a thymic remnant was resected in the 2nd surgery for recurrent primary hyperparathyroidism (PHPT).
He presented in 2013 with generalised lethargy with cCa 2.89 mmol/l, PTH 4.1 pmol/l, vitamin D 58.1 nmol/l, 24 h UCCR 0.0148. A repeat 24 h UCCR was 0.0065 (vitamin D 85.4 nmol/l). He was osteopenic and had no nephrocalcinosis. USS parathyroids and SESTAMIBI did not demonstrate any suspicion of parathyroid adenoma. CT neck also did not show any parathyroid adenoma.
From these investigations, it is possible that he has familial hypocalciuric hypercalcaemia (FHH) in view of the recurrent hypercalcaemia, histological findings and equivocal 24 h UCCR. His genetic screen for calcium sensing receptor (CASR) gene mutation is awaited.
This case illustrates the challenges of diagnosing PHPT vs FHH especially in patients with equivocal results. None of the investigations can differentiate between the two with absolute certainty.
Distinguishing between PHPT and FHH has traditionally relied upon 24 h UCCR. A 24 h UCCR of <0.01 suggests FHH (85% sensitivity and 88% specificity) whilst >0.02 was typical of PHPT. However, 24 h UCCR between 0.01 and 0.02 is a grey area.
CASR gene mutation is detected in up to 80% of patients with FHH. Although genetic analysis is still not recommended for routine evaluation of PHPT, it can be very useful in patients who have equivocal 24 h UCCR in whom FHH is suspected to avoid unnecessary surgical intervention.

DOI: 10.1530/endoabs.34.P100

P101

Efficacy and safety of doxazosin in perioperative management of phaeochromocytoma

Gautam Das, James Clark, Benjamin Field & Sunil Zachariah
East Surrey Hospital, Redhill, UK.

Case history
We present the case of a 40-year-old man who was referred to a cardiologist with hypertension. He was initiated on bisoprolol and doxazosin. Investigations revealed elevated urinary metanephrines and catecholamines, with normetadrenaline 7.4 μmol/24 h (normal <2), noradrenaline 820 nmol/24 h (normal <500) but normal dopamine levels. He was referred to our clinic. Two further 24 h urine catecholamine assays were elevated. Dexamethasone suppression test and aldosterone:renin ratio were normal. CT scan revealed a 2.8 cm right adrenal mass and normal left adrenal gland. MIBG uptake was increased in the right adrenal gland.
As patient was already on doxazosin a decision was made to continue it as the α-blockade agent, rather than swapping to phenoxybenzamine. The maximum tolerable dose was 8 mg daily. The patient was referred for laparoscopic adrenalectomy. In the preoperative assessment clinic, blood pressure was 118/70 mmHg but, during induction of anaesthesia, systolic BP rose to 300 mmHg. The operation was cancelled and an endocrine opinion was sought. Phenoxybenzamine was started in place of doxazosin and, over the next few weeks, the dose was titrated up to 40 mg twice daily. The patient subsequently underwent uneventful laparoscopic adrenalectomy in January 2013. His hypertension resolved off all medication. Histological findings were of phaeochromocytoma with no overt malignant features; genetic testing is awaited.
Discussion
Adequate α- and β-adrenoceptor blockade is essential to minimise morbidity and mortality during resection of catecholamine-secreting tumours. Phenoxybenzamine, a non-competitive α-adrenoceptor antagonist, has historically been the standard choice for perioperative management. However, recent publications have proposed that doxazosin could be a safe alternative.
Our unit has dealt with ten cases of phaechromocytoma over past year. Eight patients had a smooth pre-operative period and successful surgery on phenoxybenzamine. Two patients were α-blocked initially with doxazosin but, after similar difficulties, both were swapped to phenoxybenzamine.
We review the literature and evidence on use of doxazosin and discuss possible reasons for its failure. We are also keen to learn of experience in other centres.

DOI: 10.1530/endoabs.34.P101

P102

Are we appropriately referring patients for short synacthen testing?

Danielle Foucault[1], Sara Guthrie[2] & Parijat De[2]
[1]University of Birmingham, Birmingham, UK; [2]City Hospital, Department of Diabetes and Endocrinology, Birmingham, UK.

Aims
Short synacthen test (SST) is commonly used to diagnose adrenal insufficiency (AI), and with serum ACTH, can help differentiate between primary and secondary AI. The aim of this audit was to evaluate the clinical appropriateness of SST in our endocrine unit.
Methods
We looked at all SSTs performed between August 2012 and August 2013. Relevant clinical information was collected from patient notes and database. A SST was considered inappropriate if no reason was provided for the test or the patient's clinical history did not vaguely support the need for a SST.
Results
68 patients satisfied the study criteria and a total of 79 SSTs were conducted; nine patients had at least one repeat SST during this period (54% females, 46% males – mean age 46 years). Ethnicity: 25 - White-British, remainder Asian, Black, Others and mixed ethnicity. The most common indication for a SST referral was suspected secondary AI (25: 17 pituitary, eight exogenous steroid), followed by non specific symptom complex (NSSC) of fatigue/weight loss/hypotension/dizziness (23), hyponatremia (5), primary AI (2) and other causes (13).
24/79 (30%) SSTs were abnormal – 10, 8, 9 and 3% of the abnormal SST results were due to pituitary problems (8: empty sella – 2, prolactinoma – 2, pituitary adenoma – 4), exogenous steroid (6), NSSC (7) and others (3: 1 each of primary AI, hyponatremia and idiopathic) respectively. 7/79 (9%) SSTs were deemed inappropriate.
Conclusion
While the most common indication for a SST was suspected secondary AI (32%), 70% of initial SSTs were found to be normal. Furthermore, 9% of SST referrals were considered inappropriate. There is thus a discrepancy between clinical suspicion of AI and actual adrenal function. We would suggest careful selection of patients for SST consideration (suspected secondary AI and NSSC) so as to minimize future unnecessary SSTs in the vast majority.

DOI: 10.1530/endoabs.34.P102

P103

The use of s.c. denosumab for cases of hypercalcaemia refractory to i.v. bisphosphonate therapy

Muhammad Khan, Karen Perkins & Franklin Joseph
Countess of Chester Hospital, Chester, UK.

Despite i.v. bisphosphonates, including pamidronate and zoledronate, representing the established agents used to reduce calcium levels, a proportion of patients with hypercalcemia either fail to respond to such treatments or relapse following their use.
We present a case of hypercalcaemia refractory to conventional treatments that was treated with the use of the human MAB denosumab.

A 70-year-old male presented to the emergency department following shortness of breath due to an underlying mesothelioma requiring palliative treatment. During the admission, the patient was noted to have an elevated corrected calcium level of 3.31 mmol/l (range: 2.1–2.7 mmol/l) which was presumed to be hypercalcaemia of malignancy. Despite improving to 2.7 mmol/l with i.v. fluids and 30 mg of i.v. pamidronate, levels increased to 3.12 mmol/l within 3 days. With an adequate eGFR of $>$90 ml/min, 90 mg of i.v. pamidronate was then used to correct his calcium level but only provided a temporary benefit. Biochemical analysis of the level of parathyroid hormone revealed it was elevated at 20.7 pmol/l (range:1.6–6.9 pmol/l), thereby suggesting that this was primary hyperparathyroidism and not hypercalcemia of malignancy. Although i.v. zoledronate was then tried, within a period of a week, the patient's calcium levels had increased to 3.08 mmol/l. Given the delay in obtaining approval for cinacalcet for primary hyperparathyroidism, the recalcitrant nature of the patient's hypercalcaemia and the patients deteriorating condition, 60 mg of s.c. denosumab was trialled off-label. The patients corrected calcium levels fell to 2.76 mmol/l and remained within normal limits for over a month. The patient however succumbed to his malignancy and unfortunately passed away.

Conclusion

Denosumab may provide another therapeutic option for the management of hypercalcaemia refractory to i.v. bisphosphonate therapy. There are ongoing studies which will give us more evidence over the next few years.

DOI: 10.1530/endoabs.34.P103

P104

Localised Charcot of the Hallux

Andrzej Rys, Tania Woodrow, Harvey Chant, Kim Farmer, Michael Butler & Duncan Browne
Department of Endocrinology and Diabetes, Royal Cornwall Hospital, Truro, UK.

In patients with diabetes an isolated 'sausage toe' is suggestive of underlying osteomyelitis. Neuropathic (Charcot) arthropathy, devastating complication of diabetes, normally presents in the midfoot and can be precipitated by surgery or minor trauma.

We report a 62-year-old man with a 2-year history of well controlled type 2 diabetes (HbA1c 56 mmol/mol), with no micro or macro vascular complications, referred to diabetic podiatrist with a hot, red, swollen, left hallux (sausage toe appearance) 8 weeks following minor trauma. The temperature difference between left and right hallux was 6.0–8.1 °C. Examination of the fore and midfoot was normal, all pedal pulses were biphasic, there was objective peripheral neuropathy. Initial appearances suggested underlying osteomyelitis but blood results were not supporting this: WCC 8.5 10×9/l, CRP 2.1 mg/l. X-rays revealed fragmentation of the distal phalanx compatible with previous crush fracture but clinical suspicion of osteomyelitis remained high.

Subsequent MRI suggested fracture of the terminal phalanx of the left hallux with surrounding extra osseous calcification and ossification but no definite evidence of associated infection.

In view of normal inflammatory markers no antibiotic therapy was initiated and conservative management (off loading, foot wear) adopted. Repeat CRP and WCC stayed within the normal limits although the hallux remained swollen and warm (7.5 °C temperature difference). After 4 months X-ray appearances progressed to show destruction at the interphalangeal joint yet clinically the toe improved with reduced swelling and temperature. A diagnosis of localised hallux Charcot was made. Two months later the patient presented with classical Charcots of the right midfoot with typical radiological changes.

Localised Charcot changes of the Hallux is not widely reported but should be considered in the differential of a hot swollen hallux prior to assuming a diagnosis of osteomyelitis. Clinicians must be aware of these unusual sites of Charcot's arthropathy.

DOI: 10.1530/endoabs.34.P104

P105

Phaeochromocytomas and paragangliomas: a 20 year experience

Anand Velusamy, Vanitha Karunakaran, David Kirtchuk & John Quin
Royal Sussex County Hospital, Brighton, East Sussex, UK.

Phaeochromocytomas and paragangliomas account for about 0.1% of cases of persistent hypertension. Only 50% of these are being diagnosed as symptoms are often paroxysmal.

We report a retrospective data analysis on 16 random patients (age 24–71 years, mean 51) diagnosed and treated for phaeochromocytoma and paraganglioma in our centre over the past 20 years. Symptom duration was 6 weeks to 15 years with female preponderance 2:1 (69 vs 31% in males). Sympathetic symptoms were present at diagnosis in 50% (with hypertension), 30% (without hypertension) and as an incidental finding in 20%.

12 patients had phaeochromocytoma; all unilateral and predominantly right sided (58%). It was malignant in one patient with metastasis to liver and pelvic bone. Initial size on CT ranged from 0.3 to 10 cm (mean 5 cm). One familial case was identified with neurofibromatosis type 1 (8%), the remaining 92% being sporadic. In four patients with paraganglioma, three were malignant. The secretory pattern in phaeochromocytomas was predominantly adrenaline (67%) with combination of both adrenaline and noradrenaline in 58%. The inverse was true for paragangliomas with 100% elevated urinary noradrenaline. 24-h urine dopamine was elevated in malignancy in both conditions. 63% of total cases were MIBG avid with no uptake in six patients – benign phaeochromocytomas (3) and paragangliomas (1 benign and 2 malignant). Phenoxybenzamine was commenced during first consultation (80%) and discontinued following surgery in all patients with phaeochromocytomas. Laparoscopic adrenalectomy was performed in 67 and 33% had laparotomy. All but one patient from both groups under the age of 45 were referred for mutation analysis and none were identified. A low threshold of suspicion for the above conditions is required to initiate early treatment and avoid long-term complications and sudden death. The poor specificity of MIBG indicates need for other functional modalities at an earlier stage to diagnose, predict and influence prognosis in potentially malignant paragangliomas given the poor prognosis and uncertainty in available treatment modalities.

DOI: 10.1530/endoabs.34.P105

P106

Autoimmune adrenal insufficiency presenting as severe hypercalcaemia

Matthew Carroll[1], Angelos Kyriacou[2], Tim Cooksley[1] & Stephen McGlynn[2]
[1]Department of Acute Medicine UHSM, Manchester, UK; [2]Department of Endocrinology UHSM, Manchester, UK.

Case history

A 42-year-old female presented to the emergency department with dizziness, vomiting, abdominal pain and thirst. While investigating a 3-month history of lethargy, menstrual irregularity and weight loss, her GP had found a raised TSH, FSH and LH and had prescribed levothyroxine and, 1 week prior to admission, Adcal-D3 supplements. On examination, she was hypotensive and hyperpigmented.

Investigations

Na^+ 130 mmol/l (132–144), K^+ 4.8 mmol/l (2.5–5.3), urea 18.9 mmol/l (2.5–7.8), creatinine 275 mmol/l (40–90), Hb 87 g/l (115–165), MVC 92 fl (80–97), serum B_{12} 1062 ng/l (187–883), folate 18.3 µg/l (3.1–20.5), ferritin 183 µg/l (10–204), amended calcium 3.55 mmol/l (2.20–2.60), albumin 27 g/l (35–50), glucose 4.9 mmol/l ($<$6), random cortisol 38 nmol/l (100–500), ACTH 748 ng/l (0–46), PTH 0.5 pmol/l (1.5–9.3), TSH 10.31 mU/l (0.35–5.00), free T4 15 pmol/l (9–19), prolactin 281 mU/l (109–557), LH 16 IU/L ($>$20 post menopausal), FSH 9.0 IU/L ($>$25 post menopausal) and oestradiol 232 pmol/L ($<$103 post menopausal). Urgent SST: cortisol 23, 24 nmol/l at 0, 30 min. Positive adrenal antibodies confirmed autoimmune adrenal insufficiency.

Treatment and progress

Intravenous fluids and hydrocortisone were given with a rapid clinical and biochemical improvement. Electrolytes and renal function normalized within a week, but she remained anaemic. Her amended calcium reduced to 2.42 mmol/L within 48 h. Abdominal CT confirmed bilateral adrenal atrophy but no evidence of any neoplastic disease.

Further tests revealed

TSH 18.36 mU/l, fT4 8 pmol/l, TPO antibodies 783 IU/mL ($<$6), LH 21 IU/l, FSH 18 IU/l, oestradiol 762 pmol/l, consistent with polyglandular autoimmune syndrome type 2. Ovarian and coeliac antibodies were undetected.

Conclusions

1. Severe symptomatic hypercalcaemia is a rare but important presentation of adrenal insufficiency.
2. Treatment of hypothyroidism in undiagnosed adrenal insufficiency can worsen hypercalcaemia and precipitate an Addisonian crisis.
3. Increased intestinal calcuim absorption is recognised in untreated adrenal insufficiency and oral calcium supplementation can precipitate hypercalcaemic Addisonian crisis.
4. Treatment of the Addisonian crisis rapidly corrects hypercalcaemia, renal failure and electrolyte imbalance.
5. Extensive investigations are rarely necessary.

DOI: 10.1530/endoabs.34.P106

P107

Somatostatin analogue therapy with good biochemical response in a patient with ectopic ACTH secretion due to high grade metastatic neuroendocrine tumour

Nidhi Choudhary, Tabinda Dugal, Steven Creely, Benjamin Hopwood & Duncan Browne
Royal Cornwall Hospital, Treliske, Truro, UK.

Case
We present the case of a previously fit and well 53-year-old lady who presented with persistent hypokalaemia (1.9 mmol). She was cushingoid and testing confirmed Cushing's syndrome with random cortisol of >1750 nmol/l, ACTH 838 ng/l and non-suppressed cortisol (1099 nmol/l) after low dose dexamethasone suppression test. MRI pituitary was normal. Abdominal CT scan showed bilateral adrenal hyperplasia and large (>10 cm) mass with necrotic centre replacing the right lobe of the liver. Histology confirmed a high-grade metastatic neuroendocrine tumour. On octreotide scanning, this was intensely octreotide avid. Urinary 5HIAA (334 μg/ml) and chromogrannin A (>300 pmol/l) were markedly elevated. Her inpatient stay was complicated by type 1 respiratory failure, pulmonary oedema and pneumonia requiring ITU admission. Treatment options including hepatic resection and radiolabelled octreotide treatment were discussed extensively at the neuroendocrine MDT but she was unsuitable till her general condition improved. Despite treatment with metyrapone and then ketoconazole, her potassium remained low. She responded well to a trail of octreotide treatment (100 mg three times a day and then lanreotide 30 mg every 2 weeks). Cortisol decreased from >1750 to 156 nmol/l and ACTH from 838 ng/l to 115 pg/l after 3 weeks of treatment. She died a few months later with severe sepsis.
Discussion
Cushing's syndrome due to ectopic ACTH secretion has a high morbidity and mortality, due to underlying tumour and the sequelae of severe hypercortisolemia. Rapid treatment is mandatory. Up to 80% of ectopic ACTH-producing tumours have somatostatin receptors suggesting somatostatin analogues (octreotide) may reduce ACTH production. However, the therapeutic role of these agents is still evolving. Our patient with ectopic ACTH secretion had a good biochemical response to octreotide. Octreotide is not a first line agent to control hypercortisolemia but may be a useful agent when other inhibitors of steroidogenesis fail or other options are not appropriate.

DOI: 10.1530/endoabs.34.P107

P108

Improving the quality of endocrinology teaching to clinical medical students

Philippa Vega[2,3], Faye Gishen[2,3] & Andrew Solomon[1,2]
[1]East and North Hertfordshire NHS Trust, Hertfordshire, UK; [2]Royal Free London NHS Foundation Trust, London, UK; [3]UCL Medical School, London, UK.

Background
This project endeavoured to improve the quality of endocrinology teaching to clinical medical students through the use of a new multi-stage process.
Methods
We created a co-ordinated block containing 4-h teaching sessions (4×1 h) in which eight students in their first clinical year presented answers to questions based on clinical endocrinology cases they had seen and selected. The process started with identification of cases in the out-patient clinic, followed by self-directed learning, and presentation to the student group. A Consultant Endocrinologist assisted with deciding on one key clinical question per case and gave a real-time reflection to students' following each session A typical example was to find out reasons why a 34-year-old patient with Graves' disease, might undergo remission during pregnancy, and then relapse *post-partum*. The teaching was assessed using validated feedback using measures, co-ordinated by an objective facilitator; with quantifiable results that could be compared to other endocrine teaching and the overall student module.
Results
As a collective dataset – with all answers ranked at levels from 1 to 5, the overall total response score (of $n=120$) was 91/120, or 75.8%. This is better than the associated response for the endocrinology teaching overall (67.7%), which included other teaching styles. In addition it improved on average positive ratings (70%) than the mean for the module as a whole (55%). Therefore, not only was the new style of teaching rated higher than other clinical rotations, it was a preferred style within the speciality.
Discussion
Our novel method, as evidenced by feedback, resulted in greater interest and motivation to learn, and a greater understanding of how to devise and answer clinical questions, facilitating deep learning. Increased confidence in the key skill of presenting clinical cases was a further positive outcome to this project.

DOI: 10.1530/endoabs.34.P108

P109

An audit into the screening tests used for Cushing's syndrome

Mark Platt
University of Manchester, Manchester, UK.

Cushing's syndrome (CS), is the prolonged exposure to excess cortisol within the body and has a significant negative prognostic impact if left untreated. There are several screening tests available for CS: urinary free cortisol (UFC), overnight dexamethasone suppression test (oDST), and late night salivary cortisol test (LNSC).
This audit evaluated the screening of patients for CS by the Manchester Royal Infirmary (MRI), in 2012 against guidelines provided by The Endocrine Society and the MRI hospital policy. The parameters assessed in this audit were: were patients screened for CS adequately, Were discordant results followed up, could medication have interfered with the result and were positive results managed properly. This audit also determined how well the tests provided correlated with each other as a means to assess their consistency in screening for CS.
The MRI clinical biochemistry department collated the data for all the patients who received screening tests for CS in 2012. Each patient was then assessed, using their records on Medisec to determine how suitable their management was. The correlation between UFC and oDST tests was calculated using patients who had both tests. The SPSS program was used to calculate the κ co-efficient which quantifies the correlation.
In 2012, 80 patients were screened for CS using UFC and oDST tests and although management was suitable on the whole, patient care could be improved in some areas. Recommendations from this audit will look at ways the impact of medication can be taken into account when screening. The κ co-efficient provides insight on the UFC and oDST correlation. This audit will discuss this result and its significance with regards to patient management.

DOI: 10.1530/endoabs.34.P109

P110

Testicular adrenal axis lymphoma

Madhurima Vidyarthi, Awad Awad-Elkarim, Khalid Saja, Alison Brownell & Frederick Nkonge
Barking Havering & Redbridge Hospitals NHS Trust, Romford, UK.

77-year-old male presented with a 1 year history of lethargy, anorexia, abdominal discomfort, weight loss since September 2011. He has a background of psoriasis, primary hypothyroidism (TPO normal), cataract, glaucoma and in 2003 testicular lymphoma treated with CHOP14, intrathecal methotrexate and orchidectomy.
GP assessment showed upper abdominal fullness, mild anaemia elevated ESR, ferritin and LDH hence the haematology referral. Total body CT described massively enlarged bilateral adrenal masses, right hilar lymph lymphadenopathy. Diagnosis of adrenal dysfunction was proposed, subsequently confirmed with short synacthen test showing cortisols of 172, 193 and 174 nmol/l at baseline, 30 and 60 min (ACTH 371 ng/ml). Normal results for 24 h urine metanephrines, chromogranin A and B and adrenal antibodies.
Serum testosterone of 2.4 nmol/l, FSH (25 IU/l) and LH 22.4 IU/l) confirmed concurrent orchidectomy hypogonadism. PSA 7.2 μg/l.
There was marked improvement in his well-being, energy and appetite upon prompt hydrocortisone and fludrocortisone therapy.
CT guided adrenal biopsy histology confirmed the presence of diffuse large B cell lymphoma, similar histology to that of the 2003 testicular lymphoma.
Chemotherapy was given completed three cycles of RCHOP and followed by two cycles of RCVP (one intrathecal).
The 3 months adrenal response to chemotherapy was significant with massive adrenal lymphoma reduction. The left adrenal measured 62×18 mm (previously 97×62 mm); right adrenal 37×18 mm (previously 70×57 mm) at 3 months.
Lymphoma adrenal insufficiency is uncommon but described. This case was unusual with an 8-year gap of a recurrent lymphoma affecting two endocrine glands. Follow-up assessment demonstrated no adrenal secretory function recovery. Short Synacthen test in October 2013 results were 86, 97 and 105 nmol/l at 0, 30, 60 min, respectively. He remains fairly well.

DOI: 10.1530/endoabs.34.P110

P111

A rare case of Cushing's syndrome caused by 'cyclical' ectopic ACTH secretion

Preeshila Behary[1], Raghunath Kadiyala[1], Agnieszka Falinska[1], Hannah Beckwith[1], James Jackson[1], Catherine Mitchell[2] & Jeannie F Todd[1]
[1]Imperial College Healthcare NHS Trust, Imperial Centre for Endocrinology, Hammersmith Hospital, London, UK; [2]Hillingdon Hospital NHS Trust, London, UK.

A 61-year-old lady presented with rapid onset of lethargy and reduced mobility with inability to use stairs over 1 month. Prior to this, she was fit and well and was a lifelong non-smoker. At presentation, she was overweight and had evidence of skin bruising and severe proximal myopathy of her legs. A midnight cortisol was >1710 nmol/l with a corresponding ACTH of 610 nmol/l confirming ACTH dependent Cushing's syndrome. Serum potassium was 2.6 mmol/l and a new diagnosis of type 2 diabetes was made. She was commenced on metyrapone 500 mg tds for significant disease burden. MRI of pituitary revealed no lesion and CT scan of adrenals showed bilateral adrenal hyperplasia. In preparation for IPSS, metyrapone was stopped and cortisol levels were monitored. It was noted that her cortisol levels were consistently below 250 nmol/l with a corresponding ACTH of 34 ng/l. A midnight cortisol after discontinuation of metyrapone for 2 weeks was low at 41 nmol/l consistent with spontaneous resolution of Cushing's syndrome. Insulin Tolerance Test showed sub-optimal cortisol response and she was commenced on hydrocortisone replacement therapy. However, within 3 months since discharge, she represented to hospital with reduced mobility and hypokalaemia. A LDDST after stopping hydrocortisone, confirmed relapse with a cortisol of >1650 nmol/l at 48 h. IPSS excluded a central ACTH source and a gallium 68 DOTATATE PET CT identified a 1.6 cm gallium avid lung lesion consistent with possible ectopic source. She is awaiting resection of the lung nodule.

Learning points

i) In patients with Cushing's syndrome whose cortisol levels respond briskly to low dose metyrapone, spontaneous remission should be considered. ii) Careful monitoring of patients with Cushing's, who appear to recover spontaneously is essential as relapses are frequent. iii) This is a very unusual case as cyclical Cushing's has only once been reported in patient with ectopic ACTH production.

DOI: 10.1530/endoabs.34.P111

P112

Management of hyponatremia in secondary care

Sophie Mescall[1], Amataullah Hashi[1], Polly Begum[1] & Muhammad Ali Karamat[1,2]
[1]Heart of England NHS Foundation Trust, Birmingham, West Midlands, UK; [2]University of Birmingham, Birmingham, West Midlands, UK.

Introduction

Hyponatraemia is the most frequent electrolyte disturbance in clinical practice. It is encountered in isolation or as a complication of other medical conditions. The classification of hyponatraemia by volume status and severity – mild (125–134 mmol/l), moderate (115–124 mmol/l), and severe (<115) is of significance both prognostically and as a means of guiding management.

Methodology

A retrospective review was carried out into diagnosis and investigations of patients presenting to Acute Medical Unit at Solihull Hospital over a period of one month as either primary-care referrals for, or incidental findings of hyponatraemia. The focus of this audit was on the management of patients presenting with moderate–severe hyponatraemia, with local trust guidelines determining this as a serum sodium concentration of <125 mmol/l.

Aims

To determine whether patients presenting to AMU underwent necessary investigations and diagnostic work-up as stipulated by NICE guidelines. These investigations include: serum/urine osmolality, TFT's, urinalysis, serum cortisol, myeloma screen, CXR, fluid intake, and medication review.

Results

Over this evaluation period, 33 patients were found to be moderately–severely hyponatraemic. The majority (85%) of these patients were identified incidentally. Those presenting with severe hyponatraemia accounted for 50% of primary care referrals for hyponatraemia in comparison to incidental findings of 11%. Fifty-five percent of patients presenting with hyponatraemia were on medications exacerbating this: offending medications were suspended in 89% of these patients during their admission. The full diagnostic work-up was not used in a single patient; with a myeloma screen being the least frequently requested investigation. However an underlying aetiology was found in 70% patients, with clinical improvement in 85%, highlighting the importance of clinical judgement in requesting appropriate investigations. Four out of 33 patients (12%) died during hospital admission suggesting a high mortality rate.

DOI: 10.1530/endoabs.34.P112

P113

A case of severe hypercalcaemia

Vilashini Arul Devah, Xiang Lee, Paul Turner, Vinutha Shetty, Komal Imtiaz, Simon Howell & Kalpana Kaushal
Lancashire Teaching Hospitals Foundation Trust, Preston, Lancashire, UK.

We present the case of a 74-year-old man who was admitted with a short history of confusion and reduced mobility. He had a raised serum corrected calcium (cCa) of >5 mmol/l, serum parathyroid hormone (PTH) of 1.9 pmol/l (range 1.6–6.8 pmol/l) and acute renal failure. He was rehydrated and given a dose of i.v. zoledronate. Two days later his serum cCa remained elevated (4.39 mmol/l) with persistently impaired renal function. He underwent dialysis and serum cCa improved transiently, the nadir value being 2.94 mmol/l.

Imaging with computed tomography showed incidental acute pancreatitis, bilateral pleural thickening, and mild pulmonary fibrosis. Myeloma screen was negative. Repeat PTH levels were elevated between 99 and 205 pmol/l. Sestamibi scan was equivocal but suggestive of a left inferior parathyroid adenoma. Ultrasound showed a 1.7 cm by 1.6 cm by 3.2 cm adenoma at the inferior pole of the left thyroid gland.

It emerged that he presented to his local hospital 7 years previously with similar symptoms and a serum cCa of 5.53 mmol/l. He had received bisphosponate and i.v. fluids but refused further assessment.

A second dose of bisphosphonate (cCa >3 mmol/l) resulted in minimal lowering of serum cCa and he was commenced on cinacalcet and i.v. fluids. A third dose of bisphosphanate again resulted in only a transient improvement in hypercalcaemia. He was referred for a surgical opinion and underwent inpatient parathyroidectomy. He required further dialysis pre-operatively as his cCa was around 3.7 mmol/l. Postoperatively, serum cCa and PTH normalised. Histology of the 3.5 cm parathyroid lesion showed no evidence of malignancy.

This case highlights the clinical challenges we faced in managing this gentleman's severe hypercalcaemia. To our knowledge, there has been no case report of such resistant hypercalcaemia secondary to a parathyroid adenoma. Once the diagnosis is established, urgent referral for inpatient parathyroidectomy may reduce the morbidity associated with this condition.

DOI: 10.1530/endoabs.34.P113

P114

Baseline assessment of patients referred for 'metabolic surgery': managing the myriad of metabolic abnormalities

Hafsa Imran, George Varughese, Chandra Cheruvu, Ananth Nayak, Emily Cooper & Lakshminarayanan Varadhan
University Hospital North Staffordshire NHS trust, Stoke on Trent, UK.

Aim

Nutritional abnormalities are common after bariatric surgery and it is vitally important to assess and replace them pre-operatively to ease management post-operatively. The aim of our study was to assess the prevalence of various metabolic abnormalities at first visit to a tertiary combined bariatric endocrine clinic.

Methods

Data was collected on 200 patients referred for consideration of bariatric surgery. Being a retrospective analysis and therefore accepting the limitation of availability of all data on every patient, further statistical evaluation was done.

Results

Mean weight was 139 kg, mean BMI was 50.1 kg/m^2.

Haemoglobin <12 gm/dl in 6.1% (one microcytic and 11 normocytic).

B12 deficiency in 2% (none of these patient anaemic).

Folate deficiency in 14.5% (none of these patient anaemic).

Albumin <35 g/l in 14.5%.

Total cholesterol >5 mmol/l in 41.3%.

Alanine transaminase >40 IU/l in 29%.

Adjusted calcium deficient in 6.1%; vitamin D deficient in 41.4% (only 6% of these had low calcium); and PTH levels consistent with secondary hyperaparathyroidism in 46.0%.

Thyroid checked: 13.2% had pre-existent thyroid abnormality and 3% subclinical hypothyroidism.

Conclusion
A significant proportion of patients referred for assessment for bariatric surgery have myriad of biochemical abnormalities. Low levels of micronutrients may not manifest as hard biochemical end points in most patients. It is therefore vitally important that nutritional deficiencies and biochemical changes are actively assessed and identified, investigated and treated as appropriate in patients with morbid obesity, before proceeding with bariatric surgery.

DOI: 10.1530/endoabs.34.P114

P115

Detecting vitamin D deficiency in South Asians: is a population or targeted method better?

Samiul Mostafa, Kamlesh Khunti, Danielle Bodicoat, David Webb & Melanie Davies
University of Leicester, Leicester, UK.

Background
UK South Asians (SA) are at risk of vitamin D deficiency (VDD, defined as 25-OH vitamin D <25 nmol/l) and insufficiency (VDI, 25-OH vitamin D 25–49 nmol/l), which increases the risk of metabolic bone disease. As VDD and VDI are often asymptomatic, many individuals will be unaware of this metabolic abnormality. As there is little information on how to detect VDD/VDI in the community, we wished to investigate if they were identified more effectively using either a population or targeted method.
Methods
The VITALITY study is a randomised controlled trial of vitamin D replacement in VDD SA; the screening phase involved detecting VDD. Individuals who had not taken vitamin D and/or calcium therapies for at least two months presented to screening sessions consisting of a 25-OH vitamin D measurement, through self-referring after seeing local advertisements in magazines, internet health websites or at promoting events (population method). Secondly, primary care computer databases were searched based on criteria that increase the chances of finding VDD including BMI, waist circumstance or prediabetes (targeted method).
Results
From December 2012 to October 2013, 63 individuals were screened with a mean age of 48.5 years. The mean 25-OH vitamin D level was 32.9 nmol/l (s.d. 20.7) and 23 (36.5%) people were VDD with a further 27 (42.9%) with VDI. Only 13 (20.6%) individuals had a 25-OH vitamin D ≥ 50 nmol/l. Comparing targeted and population methods ($n=21$ and 42 respectively), there were no significant differences for mean 25-OH vitamin D levels (31.9 nmol/l (22.0) vs 34.4 nmol/l (20.8), $P=0.68$). 33.3% of people in the targeted group had VDD compared to 38.9% in the population group, $P=0.55$; for VDD and VDI combined these numbers were 80.9 and 76.2%, respectively, $P=0.53$.
Conclusion
VDD and VDI are common in SA, which is probably why the targeted method did not detect more cases than the population method.

DOI: 10.1530/endoabs.34.P115

P116

Endocrinology in a district general hospital; it's not all thyroid disease

Emily Brown[1], Momin Shah[2] & Simon Saunders[1]
[1]Warrington and Halton NHS Foundation Trust, Warrington, Cheshire, UK; [2]Royal Liverpool and Broadgreen University Hospital Trust, Liverpool, UK.

Aims
We set out to dispel the commonly held myth that endocrine practice in district general hospitals is dominated by thyroid disease. A local questionnaire revealed trainees felt thyroid disease made-up $>50\%$ of DGH referrals.
Methods
We reviewed consecutive new patient referrals to our endocrine clinic over a 2-year period. We documented the referral and outcome diagnosis and looked at the distribution of these referrals across the areas of the endocrine system. This was a retrospective electronic case note review. Diagnosis/ referral questions were noted. Concluding diagnosis also noted.
Results
Over 2 years (May 2009 – April 2011) there were 155 new patient referrals to 72 clinics. These were classified as follows:
Thyroid 34% (53/155), adrenal 4% (6/155), bone 1% (2/155), diabetes 4% (6/155), DNA/failed to attend 7% (11/155), gonad 15% (23/155), metabolic 15% (12/155), other 9% (15/155), and parathyroid 11% (17/155).
Conclusions
Whilst thyroid disease represents the single largest glandular problem of new patient referrals to this endocrine clinic, it still makes up only one third of all referrals. Given that thyroid disease is the commonest endocrine abnormality (outside of diabetes) this is not surprising. However, trainees do have a disproportionate view that all DGH endocrine practice is thyroid disease rather than the more diverse mix that is actually seen.

DOI: 10.1530/endoabs.34.P116

P117

Polycythaemia is a common side-effect of testosterone therapy, regardless of treatment mode, and careful monitoring of haematological indices is required

Rahila Bhatti, Belinda Grimmett, Maeve McCarthy, Tomas Agusttson, Barbara McGowan, Jake Powrie & Paul Carroll
Guy's and St Thomas Hospital, London, UK.

Background
Testosterone replacement is the mainstay of treatment for male hypogonadism. The most commonly reported adverse event in testosterone trials is polycythaemia. This study assessed the prevalence of polycythaemia in males receiving testosterone replacement and compared prevalence rates between different treatment preparations.
Methods
216 men were included in this retrospective observational study conducted from January 2009 to December 2012. 155 (72%) were treated with i.m. testosterone in the form of testosterone undecanoate (Nebido) or Sustanon. The remaining 61 (28%) men were treated with transdermal testosterone gel. Data were collected on haemoglobin concentrations, packed cell volumes, gonadotrophins, total serum testosterone concentrations and prostate-specific antigen (PSA) levels. Polycythaemia was defined as at least one haemoglobin concentration ≥ 17 g/dl or packed cell volume ≥ 0.505. A raised PSA was defined as >4.4 μg/l.
Results
Overall, 38 men (17.6%) developed polycythaemia on at least one blood sample during the follow-up period. The rate of polycythaemia was higher in the i.m. treatment group (19.4%) than the transdermal group (13.1%), as was peak haemoglobin concentration (15.58 vs 15.00 g/dl) though only the later was statistically significant ($P<0.05$). Overall there was a positive correlation between peak haemoglobin concentration and mean total testosterone level ($r(214)=0.138$, $P<0.05$). No relationship was found between PSA and mode of treatment or total testosterone concentration. Contrary to other studies, no association was found between development of polycythaemia and older age.
Conclusion
Polycythaemia is common in men receiving testosterone therapy, regardless of treatment modality. This risk should be weighed against the potential benefits prior to initiating therapy. Men receiving testosterone treatment should have their haematological variables monitored regularly and testosterone dose adjusted accordingly.

DOI: 10.1530/endoabs.34.P117

P118

Gastrinoma: difficult to diagnose difficult to treat

Ahmed Imran Siddiqi
University College London, London, UK.

Introduction
I present here a challenging case of gastrinoma which posed challenges in diagnosis and treatment. Gastrinomas are tumours of pancreas or duodenum secreting excessive gastrin leading to acid over secretion in stomach leading to ulcer, perforations, and diarrhoea.
Clinical case
A 77-year-old lady had been suffering from gradually worsening bouts of severe watery diarrhoea and vomiting for previous 10 years and six stones weight loss. Frequency of bouts had recently increased occasionally associated with abdominal pain, flushing, severe retching, heart burns with multiple vomiting episodes and feeling hot but no fever. Her medications (omeprazole, ranitidine, and creon) were of no help. She had a laparotomy for perforated anterior jejunal wall 3 years ago. Her BP was 152/86 mmHg, no lymphadenopathy and mild

tenderness around her epigastrium. Multiple endoscopies showed gastritis and duodenitis, biopsy confirmed oesophageal gastric metaplasia, chronic duodenitis, and intestinal metaplasia of stomach. Gastrin was mildly raised a few times; faecal elastase was very low suggesting exocrine pancreatic dysfunction. Urinary 5HIAA, TTG, selenium, zinc and C1 elastase inhibitor, thyroid function test were all normal. Small bowel meal and CT abdomen showed no abnormality except thickened lower oesophagus. Secritin stimulation test confirmed gastrinoma. Her gallium dotatate PET scan, octreotide scan and endoscopic ultrasound showed a nodule between stomach and head of pancreas which was resected but gastrin levels remained high following surgery but the bouts of diarrhoea reduced considerably. Short acting octreotide relieved all the symptoms and remains well controlled on long acting octretide now.

Conclusion

Patients with these rare tumors require systematic stepwise approach. Cure if often not achieved but significant improvement in symptoms is still very rewarding.

DOI: 10.1530/endoabs.34.P118

P119

Severe hyponatraemia, hypokalaemia and associated seizure following the administration of sodium picosulfate/magnesium citrate (picolax): a case report

John Storrow & Urmar Raja
Warwick Hospital, South Warwickshire Foundation Trust, Warwick, UK.

Introduction

Bowel preparation is known to cause minor electrolyte disturbances. There are only five reported cases of severe electrolyte disturbances following bowel preparation that have caused seizures. We report the case of a patient with severe hyponatraemia and hypokalaemia, resulting in a seizure, following the administration of picolax.

Case report

A 60-year-old female patient with no significant past medical history and taking no regular medications presented with confusion following administration of picolax for an elective colonoscopy. On arrival her GCS was 14/15 (E4, V4, and M6) but minutes later she had a tonic–clonic seizure, with no urinary incontinence or tongue biting lasting 2 min. Following this, her GCS was 9/15 (E2, V1, and M6). Laboratory tests revealed a sodium level of 119 (135–145) and a potassium level of 3.1 (3.5–5.5). A CT head did not identify any cause for the seizure. Lumbar puncture was normal. Following i.v. replacement of electrolytes, sodium was measured at 137 and potassium at 3.4. Further investigations revealed a serum osmolality of 244 (282–295), urine osmolality 508 (300–900), and a random cortisol level of 1000 (raised – time dependent). Forty-eight hours later, GCS returned to 15/15. The patient had no recollection of events from after she took the bowel preparation. There were no neurological deficits noted.

Discussion

Severe hyponatraemia, hypokalaemia and associated seizures following bowel preparation are rarely described in the literature. Four of the five cases in the literature describe patients who have pre-existing medical conditions and are taking regular medication which could have contributed to hyponatraemia. We urge care to be taken when prescribing bowel preparation; particularly in those with pre-existing medical conditions and taking medications which can cause hyponatreamia, and to council patients when prescribing bowel preparation on the side effects.

DOI: 10.1530/endoabs.34.P119

P120

Bilateral adrenal hyperplasia: a rare cause of Cushing's syndrome in chidren

Akshatha Taranath Kamath, Shiny Goyal Mandliya, Sanjay Rao & Sampath Satish Kumar
Narayana Hospitals, Bangalore, Karnataka, India.

Cushing's syndrome (CS) is a rare endocrine problem in children. Most of the cases in children are iatrogenic in nature due to extensive usage of topical, inhaled and oral corticosteroids. Endogenous hypercortisolism in children is mainly because of ACTH-dependent pituitary adenomas and constitutes ~70–80% of CS in children. ACTH-independent CS is rare in children above 5 years of age. Here, we are presenting a rare case of ACTH-independent macronodular adrenal hyperplasia.

A 7-year-old Iraqi boy presented to our OPD with a few years history of increased body weight, growth failure, facial swelling, and a few months history of pubic and facial hair growth and hypertension. On examination, weight 45 kg, height 114 cm, he had moon–like face, buffalo-hump, scanty terminal hair over face and pubic area and pinkish purple striae over abdominal wall. Blood pressure was high and he was on three antihypertensives. Biochemistry revealed low ACTH and high cortisol, non-suppression of cortisol on ODST. CT scan revealed bilateral bulky adrenals with nodular hyperplasia with one large lesion on the left side. Initially, he underwent laparoscopic left adrenalectomy. Post-operatively, he had persistently high cortisol with suppressed serum ACTH. Subsequently, he underwent right adrenalectomy. Post-surgery, he had very low serum cortisol. He was started on maintenance dose of oral hydrocortisone and fludrocortisone. All anti-hypertensives were stopped. He lost around 10–12 kg body weight 3 months post-surgery.

Conclusion

CS can cause serious long-term morbidity and effect the physical and mental development of the child. Treating physician can prevent these problems if he has high index of suspicion regarding endocrine problems whenever the child has obesity, high BP, and growth failure.

DOI: 10.1530/endoabs.34.P120

P121

Micrometastases in a paraganglioma

Fareeda Tabassum & Belayat Hossain
Queens Hospital, Romford, UK.

Background

PTHrP related hypercalcemia is a common cause of humoral hypercalcemia of malignancy associated with carcinoma of the lung, prostate, and breast and uncommonly in haematological malignancies and malignant pheochromocytoma. We present a case with an unusual finding of micrometastases within a benign paraganglioma and PTHrP release from micrometsatses.

Case presentation

72 years old lady presented with tiredness, poor appetite, generalized body aches, and constipation. She had a past history of hypertension, hypercholesterolemia, and carcinoma of left breast treated 5 years ago, no recurrence and was discharged a month before her current presentation. Staging CT scan at diagnosis of breast cancer showed a left adrenal mass 3.8×3.3 cm which remained unchanged on follow-up images. 24 h urine catecholamines at that time were normal.

Investigations

FBC, U&E, CXR normal. Corr Ca. 3.91 mmol/l, PO4 0.79 mmol/l, ESR108 mm/h, and PTH 0.3 pmol/l. Protein electrophoresis, TSH, prolactin, ACE, and vitamin D normal. CT scan showed left mastectomy and left adrenal mass which remained unchanged. Bone scan showed no metastases, USS parathyroid glands normal, urinary metadrenaline 5895 nmol/24 h, normetadrenaline 3997 nmol/24 h, and 3-methoxytyramine 1086 nmol/24 h. PET scan showed left adrenal mass. MIBG scan showed increased activity in left adrenal gland. PTHrP elevated 31.9 pmol/l. Patient treated for pheochromocytoma but hypercalcemic again few days after adrenalectomy. Histology showed paraganglioma with micrometastases from previously treated breast cancer. Micrometastases also confirmed on bone marrow biopsy.

Discussion

Adrenal incidentaloma on staging CT scan diagnosed as adenoma was a paraganglioma. The delay in diagnosis of paraganglioma caused complications and the presence of paraganglioma mislead the team as the likely cause of hypercalcemia which caused further delay in diagnosis of micrometastatses.

Conclusion

Not all adrenal incidentalomas are adenomas. The proportion of pheochromocytoma among incidentalomas has been reported upto 11%. As many of these individuals have no typical clinical symptoms, a careful biochemical evaluation is necessary by the endocrinologists to avoid a delay in diagnosis and complications.

DOI: 10.1530/endoabs.34.P121

P122

Mitotane ameliorates testostoxicosis due to metastatic Leydig cell tumour

Nicholas Johal[1], Michael Cullen[2], Peter Guest[3], Emilio Porfiri[2] & Wiebke Arlt[1]
[1]School of Clinical and Experimental Medicine, Centre for Endocrinology, Diabetes and Metabolism (CEDAM), University of Birmingham, Birmingham, UK; [2]Department of Oncology, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK; [3]Department of Radiology, Queen Elizabeth Hospital, University Hospitals Birmingham NHS Foundation Trust, Bimingham, UK.

Leydig cell tumour (LCT) is a stromal testicular tumour comprising 3% of testicular neoplasms. Metastases are rare, have a poor prognosis and can appear many years after tumour removal. Current therapy for metastatic disease is limited, with no role for radiotherapy and poor efficacy of chemotherapy regimens. About 50–70% of metastatic LCT show associated steroid excess, comprising not only androgens but sometimes steroids physiologically produced in the adrenals. Here we report the case of a 51-year-old man who presented with disseminated metastatic LCT deposits in liver, lung, and retroperitoneum after an orchidectomy 15 years previously. His serum testosterone was highly elevated at 93 nmol/l (normal 7–29); his 24-h urinary androgen metabolite excretion (androsterone + etiocholanolone) was 101 476 μg/24 h (normal 1487–7957). This was associated clinically with significantly impaired well-being due to restlessness, insomnia, reduced concentration, increased aggression, redness of the face and increased body hair growth. Prognosis was assessed as poor and following detailed discussions the patient opted against chemotherapy. In a palliative approach we decided to initiate the adrenolytic agent mitotane in an attempt to improve the testotoxicosis. His clinical signs and symptoms improved within a few weeks of treatment initiation and prior to reaching therapeutic mitotane levels (14–20 mg/l after 5 months of treatment); he returned to full-time work and enjoyed normal quality of life. CT follow-up imaging 6 months after starting mitotane showed stable disease. Concurrently, his urinary androgen metabolite excretion dropped from 101 476 to 12 827 μg/24h. However, after 10 months of mitotane treatment he died suddenly of a suspected cardiac arrest. Review of the literature identified four previous reports on mitotane therapy in LCT for >2 months, all reporting tumour response and reduction in steroid excess. LCT cells may share steroidogenic properties with adrenal cells and thus mitotane treatment for metastatic LCT appears to be a valid palliation option.

DOI: 10.1530/endoabs.34.P122

P123

Hypomagnesaemia during proton pump inhibitor therapy causing functional hypoparathyroidism

Senthilkumar Krishnasamy, Vineet Abbott, Rajendran Bellan Kannan, George Varughese, Ananth Nayak, Laks Varadhan & Biju Jose
UHNS, Stoke-on-Trent, UK.

Proton pump inhibitors (PPI) are widely prescribed. PPI-induced diarrhoea and hypomagnesaemia are well-documented in literature. Hypomagnesaemia is well-known to cause functional hypoparathyroidism. We describe a patient who had transient profound hypoparathyroidism which improved on discontinuing PPI and normalising severe hypomagnesaemia.

A 72-year-old male, who was on oral anti-diabetic medications, calcium/vitamin D3 supplements and PPI, presented with four weeks of fatigue, weight loss, and loose stools. He was dehydrated and had proximal muscle weakness. Other system examination was unremarkable. Serum magnesium was 0.36 (0.7–1.0 mmol/l). Admission calcium was 2.71 (2.2–2.6 mmol/l), but normalised next day on rehydration and stopping calcium/D3 supplements. Subsequent parathyroid hormone (PTH) was <0.3 (0–9.2 pmol/l). Haematinics, thyroid/liver functions, creatinine and faecal elastase were normal. Stool culture did not grow any organisms. Myeloma and coeliac screens were negative; CT chest/abdomen/pelvis was unremarkable.

Lansoprazole, a PPI, was replaced with ranitidine. Initially, he required intravenous magnesium supplementation. His general condition improved over the next few days with nutritional and electrolyte supplementation; diarrhoea settled and magnesium normalised. At review in out-patient clinic 3 months later, he was diarrhoea-free, had normal magnesium (0.73), calcium (2.56), and PTH (2.18), and was able to walk independently.

Intracellular magnesium depletion impairs the ability of the parathyroid cells to secrete PTH, often resulting in hypocalcaemia. The rapidity with which serum PTH rises in response to magnesium therapy in these patients reflects a defect in PTH secretion rather than its biosynthesis. This case is important to highlight the effect of PPI on magnesium and consequently on the parathyroid–calcium axis. This patient did not become hypocalcaemic because of the calcium supplementation. PTH was undetectable even after normalising calcium, but improved on stopping PPI therapy and correcting magnesium. Patients on long-term PPI should be warned of these side-effects and monitored periodically for electrolyte imbalances.

DOI: 10.1530/endoabs.34.P123

P124

Anti-GAD antibody encephalitis: a potentially treatable syndrome

Senthilkumar Krishnasamy, George Varughese, Adnan Al-araji, Laks Varadhan, Ananth Nayak, Indira Natarajan & Biju Jose
UHNS, Stoke-on-Trent, UK.

Autoimmune encephalitides are being increasingly recognised as important and potentially reversible non-infectious causes of the encephalitic syndrome. Myriad of clinical presentation and lack of symptom specificity leads to a wide differential diagnosis. Failure to diagnose the correct aetiology of an encephalitic syndrome can lead to significant morbidity and mortality. A 57 years old right handed female with no significant medical problems initially presented with left sided limb weakness, difficulty in walking, speech, memory disturbance, ataxia, and headache. She had marked truncal, finger nose and heel/shin ataxias and her symptoms were chronic at the time of presentation. Her left plantar reflex was extensor response. She had bulbar and cerebellar dysarthria. Lumbar puncture showed normal protein (0.53 g/l) and glucose (3.4 mmol/l). The CSF was acellular. Oligoclonal band and CSF serology was negative. Serum copper, ceruloplasmin, AFP, folate, B12, thyroid function, immunoglobulin profile, coeliac screen, paraneoplastic antineuronal and antipurkinje cell antibodies, blood film were all normal. MRI brain and whole spine cord showed white matter T2 hyperintense lesions in the brain and excluded cerebrovascular disease. CT chest/abdomen/pelvis and PET scans were normal. Anti-GAD antibody level in blood was 2 000 000 IU/ml (0–10), anti-GAD antibody level in the CSF which was 28 000 IU/ml. The ratio between CSF and blood anti-GAD antibody was 0.9. In view of significantly high titre of anti-GAD antibodies, autoimune encephalitis was suspected. She was treated with plasma exchange and intravenous immunoglobulins. Over next 12 weeks her speech, gait, and truncal ataxia improved significantly. Our case highlights the need for aggressive, early and appropriate treatment of patients with autoimmune encephalitis to achieve good outcomes. It is a disease process that if considered early, diagnosed promptly and treated appropriately (including aggressive treatment with therapies such as high-dose steroids, IVIG, plasma exchange, rituximab and cyclophosphamide) can be reversed and the patient restored to their premorbid state.

DOI: 10.1530/endoabs.34.P124

P125

Intravascular pituitary invasion by large B-cell lymphoma is a rare cause of SIADH and hypopituitarism; a case report

Marc Aitken[1], Simeen Akhtar[2] & Edward Jude[1]
[1]Tameside General Hospital, Manchester, UK; [2]Southport and Omskirk Hospital NHS Trust, Merseyside, UK.

Hyponatraemia is a very common electrolyte abnormality with varied presenting features depending on the underlying cause. The authors report the case of a 75 years old, previously fit, gentleman who presented with weight loss, lethargy, and blackouts. He was admitted to the hospital repeatedly under the general physicians over an 8 month period.

Investigations revealed persistent hyponatraemia (serum sodium 113–120 mmol/l, serum osmolality 258 mOsm/kg, urine osmolality 393 mOsm/kg, urinary sodium 40 mmol/l) consistent with a diagnosis of syndrome of inappropriate antidiuretic hormone secretion (SIADH), macrocytic anaemia and partial hypopituitarism (with secondary hypothyroidism, low testosterone and gonadotrophins and a reduced GH response as demonstrate by a diminished glucagon stimulation response). Cortisol response was normal. CT scan thorax, abdomen, and pelvis did not demonstrate any underlying cause and MRI pituitary was normal. He was commenced on thyroid, testosterone and GH replacement by the general medical team. This was rationalised to levothyroxine replacement by the endocrine team. All other investigations that were performed

failed to identify the underlying cause. Despite best management of his hyponatraemia his condition continued to worsen and he subsequently died two weeks later. A post-mortem was then performed and a diagnosis of intravascular large B-cell lymphoma was made with intravascular infiltration of the pituitary gland.
The authors recommend that endocrinologists should be involved at the outset in the management of patients with persistent hyponatraemia. Intravascular large B-cell lymphoma should be considered as a rare differential diagnosis of hyponatraemia. A literature review identified a similar case where rituximab was used in the treatment of large B-cell lymphoma resulting in a gradual resolution of hypopituitarism. This highlights the potential benefit of early diagnosis and management in the setting of SIADH associated with malignancy.

DOI: 10.1530/endoabs.34.P125

P126

Double trouble: pseudo-phaeochromocytoma in a patient with adrenocortical cancer

Christine May[1], Vidhya Jahagirdar[1], Vasileios Chortis[1,2], David Jenkins[3], Wiebke Arlt[1,2] & John Ayuk[1]
[1]Department of Endocrinology, Queen Elizabeth Hospital, University Hospital Birmingham NHS Foundation Trust, Birmingham, UK; [2]School of Clinical and Experimental Medicine, Centre for Endocrinology, Diabetes and Metabolism (CEDAM), University of Birmingham, Birmingham, UK; [3]Centre for Diabetes and Metabolism, Worcester Royal Hospital, Worcester, UK.

A 34-year-old male with no significant past medical history presented with severe abdominal pain. On detailed questioning his symptoms included tremors, headache, sweating, and agitation. Despite recent weight gain he had obvious muscle wasting. On admission blood pressure was 166/107 mmHg. Abdominal examination revealed a palpable left upper quadrant mass.
CT scan demonstrated a 15 cm mass in the left supernal region with extensive signs of haemorrhage in and around the tumour. Endocrine work up documented increased 24-h urine normetadrenaline (5.05 μmol/24 h, normal 0–3.8) and 24-h urinary free cortisol (1754 nmol/24 h, normal <130). Serum cortisol failed to suppress overnight after 1 mg dexamethasone (687 nmol/l, normal <50). The initial working diagnosis was of a phaeochromocytoma co-secreting catecholamines and ACTH.
In view of his hypertension and elevated catecholamines he was initially managed with phenoxybenzamine followed by Verapamil for rate control (intolerant to β-blockers). Despite four antihypertensive agents hypertension persisted. Later, suppressed plasma ACTH was discovered, excluding the rare entity of an ACTH-producing phaeochromocytoma. Surprisingly, MIBG imaging demonstrated no uptake in the area of adrenal mass. Plasma catecholamines repeated 3 months after initial presentation had normalised. Phenoxybenzamine was discontinued. Steroid analysis confirmed persistence of Cushing's and documented increased levels of DHEAS, androstenedione, 17-OH progesterone and progesterone consistent with the diagnosis of adrenocortical carcinoma.
The patient underwent left adrenalectomy without complications and was commenced on hydrocortisone perioperatively. The adrenal mass weighed 2195 g, measuring 21×16×10 cm. Histology confirmed adrenocortical carcinoma with a Ki-67 of 4.4% and modified Weiss score of 6/7. He will be commenced on mitotane.
Unilateral adrenal haemorrhage causing a pseudo-phaeochromocytoma presentation can occur in the context of adrenal pathology such as adrenal adenoma, adrenal carcinoma or metastatic disease. We present an unusual case of adrenocortical carcinoma secreting cortisol complicated by transient increase in catecholamines due to adrenal haemorrhage into the tumour.

DOI: 10.1530/endoabs.34.P126

P127

Audit on investigations and diagnosis of hyponatremia in in-patients at a district general hospital

Niruthika Sithamparnathan, Rebecca Dooley & Isha Malik
North Manchester General Hospital, The Pennine Acute Hospitals NHS Trust, Manchester, UK.

Background
Hyponatremia is associated with increased, inpatient mortality and length of stay and correct management necessitates doing appropriate investigations for accurate diagnosis of the cause.
Aim
The aim of this audit was to identify, the number of medical and surgical patients admitted with hyponatreima over a 4-week period; number of cases with mild (<135) vs moderate to severe cases of hyponatremia (<130 mmol/l); determine if hyponatremia was investigated and the number of cases of moderate to severe hyponatraemia, where aetiology was established, enabling us to compare our practice with current best practice – Clinical Resource Efficiency Support Team (CREST) guideline.
Methodology
All ($n=283$) patients with hyponatremia (Na <135 mmol/l) admitted to the medical assessment unit (MAU, $n=203$) and surgical triage unit (STU, $n=80$) during the month of November 2012, were identified. The audit focused on patients with serum sodium levels <130 mmol /l. All relevant clinical and biochemical parameters were collected by looking at electronic records including discharge letters and online biochemistry results.
Results
The prevalence of all degrees of hyponatraemia was 30% with the prevalence of moderate-severe hyponatremia being 8.6%.
In patients with Na <130 mmol/l – only 11% had any investigation done to delineate the underlying cause. Only in 23% of patients was an underlying aetiology identified. The average length of stay was nearly 3- and 1.5-days longer in medical and surgical patients as compared to other patients admitted in November 2012, in MAU and STU respectively.
Conclusion
The prevalence of hyponatremia was high, however the entity seemed to be under recognised and poorly investigated, leaving potential for inappropriate treatment, adversely affecting outcome and increasing length of stay.
An easy to use protocol, to appropriately investigate and manage hyponatremia has since been introduced and awareness of its existence created. The plan is to conduct a re-audit, to assess the impact of this protocol.

DOI: 10.1530/endoabs.34.P127

P128

Three cases of octogenarians with Cushing's syndrome

Jodie Sabin, Urvi Popli, John Gibson, Alex Kraus, Catrin Searell & Tony Wilton
Betsi Cadwaladr University Health Board, Bangor, UK.

Cushing's syndrome is a rare disease with an incidence of 0.72–2.4/million per year. Approximately 14% of patients are over 60 years of age but <1% are over 80 years of age. In a 2-month period, we diagnosed three females over 80 years of age as having Cushing's syndrome. None of them had a typical cushingoid appearance.
Patient 1, 80 years of age. Presentation: coincidental finding of bilateral adrenal hypertrophy on CT scanning as part of investigations for weight loss. No evidence of malignancy was found. Clinical features: hypertension, skin changes (previously attributed to treatment with inhaled and oral glucocorticoids). Co-morbidities: severe chronic obstructive pulmonary disease. Investigations: biochemical and imaging evidence confirmed a diagnosis of Cushing's disease.
Patient 2, 80 years of age. Presentation/clinical features: hypertension, peripheral oedema, and hypokalemia. Co-morbidities: none. Investigations: metastatic adrenocortical carcinoma with biochemical evidence of tumour secretion of cortisol and aldosterone confirmed.
Patient 3, 81 years of age. Presentation/clinical features: presented to acute medical unit with hypertension and dyspnoea. Recent diagnosis of type 2 diabetes. Proximal myopathy found on examination. Co-morbidities: severe aortic stenosis and stage 3 chronic kidney disease. Investigations: biochemical and imaging evidence confirmed Cushing's disease.
It is very unusual to diagnose three octogenarians with Cushing's syndrome in such a short period of time. All 3 patients had capacity to be involved in the decisions made on their management. Patient 1 was deemed too frail for any treatment other than symptomatic. Patient 2 declined treatment other than spironolactone. Patient 3, metyrapone treatment being considered.
When the rare diagnosis of Cushing's syndrome is made in elderly patients the aetiology and co-morbidities make treatment very difficult. The decisions made on treatment in these three patients was helped by their ability to contribute to the process.

DOI: 10.1530/endoabs.34.P128

P129

Spontaneous hypoglycaemia induced by ACE-inhibitor: a case report

Kah Fai Wong, Varadarajan Baskar & Rajeev Raghavan
Wolverhampton Diabetes Centre, New Cross Hospital, Wolverhampton, UK.

Background
Hypoglycaemia is rare in people without diabetes. Certain medications are known to cause hypoglycaemia in non-diabetic patients.
Case details
A 65-year-old man with background congestive cardiac failure, previous pulmonary TB (1997), bronchiectasis and pulmonary fibrosis (long-term oxygen therapy) was admitted for exacerbation of bronchiectasis. He completed a course of antibiotics and steroid treatment. While awaiting discharge, he had two episodes of spontaneous hypoglycaemia (lowest blood glucose being 2.2 mmol/l on venous sample). He was referred to the Endocrine team at this point. On further questioning, he admitted similar episodic 'funny turns' associated with sweating and feeling faint over the previous 18 months. These episodes occurred in the morning, up to three times per month with no precipitant or prodrome and resolved spontaneously after about an hour. Consumption of carbohydrates seemed to aid symptom resolution.
Investigations
Subsequent inpatient investigations including short synacthen test and coeliac screen were normal. He also underwent a prolonged fast which was abandoned by the patient after 48-h with no further recorded hypoglycaemia as an inpatient. Thus it was not possible to measure insulin or C-peptide level. He was discharged from hospital with a glucometer. In the outpatient clinic, he reported further episodes which corroborated with low self-monitored glucose readings. A thorough medication review confirmed that his symptoms were contemporaneous with the commencement of the anti-hypertensive drug Ramipril. This prompted substitution of his Ramipril to Losartan. Since then he has returned to two clinic reviews over 5 months period and has not reported any further hypoglycaemic episode. He has not required a repeat 72-h fast.
Conclusion
We report a case of drug induced hypoglycaemia in a non-diabetic patient caused by Ramipril. There are few such reported cases in the literature. Our case illustrates the importance of considering rare drug side-effects with unusual presentations.

DOI: 10.1530/endoabs.34.P129

P130

The role of genetic analysis in the diagnosis of familial hypocalciuric hypercalcaemia

Zana Ameen & Jayadave Shakher
Heart of England NHS Foundation Trust, Birmingham, UK.

Familial hypocalciuric hypercalcaemia (FHH) is a benign condition, occurs as a result of inactivating mutation in the calcium sensing receptor (CASR) gene and is autosomal dominant.
We present a 56-year-old lady with periodic mild hypercalcaemia since 2004. Her highest corrected calcium concentration was 2.76 mmol/l (NR 2.2–2.6). She was asymptomatic. Her parathyroid hormone concentration was also mildly elevated at 9.7 pmol/l (NR 1.6–6.9). Her urinary calcium output was 3.96 mmol (NR 2.5–7.5).
She had parathyroidectomy for suspected primary hyperparathyroidism (PHPT) in 2008 (two glands removed – no adenoma on histology). She was on vitamin D supplements and her last vitamin D level was normal at 71 nmol/l (NR $>$49.9 nmol/l). She was referred to our hospital in April 2013 for persistent mild hypercalcaemia. She remained asymptomatic.
Her 23-year-old son has also been diagnosed with PHPT at a different hospital and he was listed for parathyroidectomy as well. He too had mild hypercalcaemia with the highest value of 2.72 mmol/l and highest corrected value of 2.64 mmol/l. His PTH level was 5.2 pmol/l and his urinary calcium output was very low at 0.21 mmol.
We suspected the diagnosis of FHH in view of their mild biochemical derangements, low urinary calcium and family history. We contacted the other hospital and asked them to perform genetic testing which later confirmed that the mother and the son were heterozygous for c.61G$>$A (p.Gly21Arg) CASR variant. This gene has been reported in the literature to be associated with FHH.
This case illustrates the importance of making the correct diagnosis of FHH by genetic testing in order not to label these younger patients as hyperparathyroid and wrongly referring them for surgery. We have shown here that we saved a young person from having a surgical operation unnecessarily and without any proven benefits.

DOI: 10.1530/endoabs.34.P130

P131

Epidural abscess as a complication of infected diabetic foot ulcers: a case report.

Marc Aitken, Abdelaziz Marzoug & Edward Jude
Tameside General Hospital, Manchester, UK.

A 59-year-old gentleman presented with a 6-month history of painless, progressive lower limb weakness. There was no history of trauma or weight loss although he had been suffering night sweats. He had been bed bound for 4 weeks prior to admission. He was an insulin-dependent type 2 diabetic.
Examination revealed marked symmetrical wasting of the leg muscles with reduced power (3/5) and absent reflexes and plantar reflexes were up-going. He was in urinary retention. He had established peripheral neuropathy complicated by diabetic foot ulceration managed under the care of the GP and practice nurses for the previous 8 months. The ulcers appeared infected; a wound swab grew methicillin-sensitive staphylococcus aureus. Inflammatory markers (white cell count (WCC) and C-reactive protein (CRP)) were high. CT head revealed no cause for the limb weakness. He was catheterised, started on oral antibiotics for his foot wounds and transferred to the diabetic ward.
He had an MRI whole spine performed which showed a paraspinal abscess at the level of C6–T1 and epidural collection from T6 to T9 with associated spinal cord abscess. He was started on high dose i.v. flucloxacillin and discussed urgently with the Neurosurgical team who recommended continuing antibiotics and interval MRI scanning. His foot ulcers were managed by the High Risk Diabetic Foot Team. Six weeks later he was ambulant with a walking stick, his CRP and WCC were improving and scans revealed some resolution of the spinal collections. He was discharged home on oral flucloxacillin with follow-up under the Neurosurgeons and ongoing podiatric care.
Epidural and spinal cord abscess is a rare complication of infected diabetic foot ulcers. *S. aureus* is the most common organism. Prompt assessment ($<$24 h) of new diabetic foot wounds by expert practitioners now forms part of the recommended framework of care from NICE.

DOI: 10.1530/endoabs.34.P131

P132

Alternating high and low TSH levels post subacute thyroiditis

Marie-France Kong[1,2] & Rafik Karmali[2]
[1]Department of Diabetes and Endocrinology, University Hospitals of Leicester NHS Trust, Leicester, UK; [2]Department of Medicine and Endocrinology, Centre Hospitalier Universitaire Brugmann, Brussels, Belgium.

A 43-year-old African lady initially presented in September 2007 with hypothyroidism (fT_4 0.6 ng/dl (0.8–2.0) and TSH 110 mU/l (0.3–4.00)). She gave a history of pain in the neck with transient symptoms of thyrotoxicosis. It was felt that she had gone hypothyroid following an episode of subacute thyroiditis and was started on L-thyroxine. Her compliance was questioned as her TSH fluctuated from being high to being suppressed on 25 μg of L-thyroxine a day. The L-thyroxine was stopped in September 2009 when TSH remained suppressed. In January 2010 fT_4 was 1.9 ng/l, fT_3 4.60 pg/ml (2.10–4.00), and TSH $<$0.03 mU/l. Thyroid stimulating immunoglobulin (TSI) was 15.9 U/l ($<$1.5). A diagnosis of Graves' disease was made with the presence of both TSH receptor stimulating (TSAb) and blocking (TBAb) antibodies and she was started on thiamazole 10 mg a day. She was lost to hospital follow-up after December 2010 (when fT_4 was 1.7 ng/dl, fT_3 3.7 pg/ml, and TSH $<$0.03 mU/l). She was re-referred in January 2013. She was still taking 10 mg of thiamazole a day. TSH was still suppressed and her GP queried compliance and wondered whether she should be considered for radioiodine treatment. A repeat blood test showed TSH $<$0.03 mU/l, fT_3 5.0 pg/ml, and TSI 12.4 U/l. The thiamazole dose was increased to 30 mg a day. In May 2013 her TSH was 13.54 mU/l, fT_4 0.6 ng/dl, and fT_3 1.8 pg/ml. Thiamazole was reduced to 10 mg a day and stopped in July when TSH was 2.33 mU/l. She was restarted back on 10 mg of thiamazole a day in August 2013 when TSH $<$0.03 mU/l and fT_3 5.50 pg/ml. Switching between TBAb and TSAb (or *vice versa*) occurs in unusual patients after L-thyroxine therapy for hypothyroidism or anti-thyroid drug treatment for Graves' disease.

DOI: 10.1530/endoabs.34.P132

P133

Pituitary apoplexy during a second pregnancy in a woman with prolactinoma

MonicaLivia Gheorghiu[1,2], Andra Caragheorgheopol[2], Anda Dumitrascu[2] & Virgil Ionescu[3]
[1]'Carol Davila' University of Medicine and Pharmacy, Bucharest, Romania; [2]'C.I. Parhon' National Institute of Endocrinology, Bucharest, Romania; [3]ProVita Diagnosis and Treatment Centre, Bucharest, Romania.

Introduction
Pituitary apoplexy is a rare syndrome due to hemorrhagic infarction of a pituitary adenoma. It may be clinically overt (sudden, severe headache, visual disturbances, impairment in pituitary function, and altered consciousness), or subclinical. Pregnancy is a rare predisposing factor, which may raise difficulties in the diagnosis and treatment.
Case presentation
A 25 years woman diagnosed with a 1/0.8 cm prolactinoma, initial serum PRL 61.5 ng/ml (normal 1.3–24.2), without macroprolactin, in whom menses recovered only after increasing cabergoline (CAB) treatment to 1.5 mg/week. After 1 year PRL was 20.7 ng/ml, the tumor size was stable (1.1/0.9 cm) and the patient got pregnant. In the 8th week of pregnancy the patient developed a transient right-sided headache for 2 weeks. CAB was gradually tapered to 0.25 mg/week then withdrawn in the 9th month of pregnancy. Six months after delivery PRL = 59 ng/ml, tumor size was increased 1.46/0.9 cm. CAB was re-started, 3 mg/week being needed to normalize PRL. The patient got a second pregnancy, while PRL was 29.1 ng/ml and the tumor 1.24/0.94 cm. In the 8th week of pregnancy a severe frontal headache associated with fatigue and nausea, but a not visual defect, occurred for 1 week during an upper respiratory viral infection, and was ameliorated with acetaminophen. Morning serum cortisol, TSH, and FT_4 were normal. CAB was tapered and withdrawn after the 31st week of pregnancy. Three months after delivery PRL was 59.6 ng/ml and MRI showed a 1.8/1.3 cm pituitary tumor with a fluid collection suggesting a tumor haemorrhage in a late subacute stage. CAB 2 mg/week was initiated and, despite another transient 3 h-severe headache, the tumor decreased to 1/1 cm, with no visual or pituitary function defects.
Conclusion
Although a rare event, pituitary apoplexy should be considered when severe headaches occur during pregnancy in a woman with a pituitary adenoma.

DOI: 10.1530/endoabs.34.P133

P134

Endocrine considerations in ever-improving HIV-related mortality outcomes: a clinical perspective

Sarah Mensforth[1], Biju Jose[2], George Varughese[2], Neena Bodasing[2], Gurdeep Singh[1] & Kieran Fernando[1]
[1]Staffordshire and Stoke on Trent Partnership NHS Trust, Stoke-on-Trent, UK; [2]University Hospital North Staffordshire, Stoke-on-Trent, UK.

Since the advent of effective combined antiretroviral therapy (ART), mortality due to HIV disease has significantly declined. Changing trends in HIV-associated morbidity is surfacing, firstly due to the effect of HIV on virtually every human organ system and secondly due to improved life expectancy and consequent development of natural co-morbid illnesses. HIV associated endocrinopathies and metabolic diseases pose a significant disease burden in these patients. We discuss three patients with HIV infection, well controlled on ART, presenting with diverse endocrine involvement.
Young woman on ART presents with non-specific aches. Vitamin D deficiency and Tenofovir use known to affect bone mineral density (BMD) resulted in a DEXA bone scan, which showed *T*-score at −1.5. Vitamin D replacement and change to bone-friendly Abacavir stabilised the BMD at 18 months.
Middle-aged man on ART has triglyceride levels of 45 (0.8–1.8 mmol/l) and lipemic serum. Patient's poor life-style factors were addressed and started on statins with good improvement in lipids. ART-related dyslipidemia is difficult to treat; life-style measures and modification of ART along with lipid-lowering drugs are important.
Middle-aged man previously well controlled on ART has unexplained drop in CD4 counts. This prompted several investigations including random cortisol, which was low. A short Synacthen test confirmed adrenocortical deficiency. Other pituitary axes and MRI scan of pituitary were normal. He is now on hydrocortisone replacement.
HIV associated endocrine/metabolic dysfunction, causing adverse bone metabolism, adrenal insufficiency, lipodystrophy, insulin resistance, and dyslipidaemia, has heterogeneous mechanism and pathogenesis. HIV itself and opportunistic infections can directly affect endocrine organs. Endocrine gland infiltration by lymphoma and Kaposi's sarcoma is also recognised. ART is also implicated in metabolic dysfunction, mitochondrial toxicity being a key mechanism. Physicians caring for these patients should be vigilant in identifying and managing these in a timely manner to limit the associated morbidity.

DOI: 10.1530/endoabs.34.P134

P135

Next generation sequencing approach for molecular genetic diagnosis of familial partial lipodystrophy

Nihal Thomas, H S Asha, Aaron Chapla, V Manika & Shrinath Shetty
Christian Medical College, Vellore, India.

Background
Familial partial lipodystrophy type 2 (FPLD2) is an autosomal dominant inherited form of lipodystrophy due to mutation in the LMNA gene on chromosome 1q22. It is characterized by selective loss of subcutaneous adipose tissue from the limbs and trunk, with accumulation of fat in the neck and face, insulin resistance, diabetes mellitus and dyslipidemia.
Objective
To study the clinical features and establish the molecular diagnosis of two subjects with FPLD2.
Subjects and methods
Utilizing next generation sequencing (NGS) we carried out mutational analysis of LMNA gene in a woman and her mother with FPLD2 phenotype.
Results
A 23-year-old lady and her mother were diagnosed to have diabetes mellitus at age 16 and 30 years respectively. Both never had keto-acidosis and required high doses of insulin with suboptimal glycaemic control. They had cushingoid appearance of the face, acanthosis nigricans, minimal fat in the limbs with prominent muscular contours and veins, and hepatomegaly consistent with FPLD2. The subject and her mother had elevated HOMA-IR and hypertriglyceridemia suggestive of insulin resistant diabetes mellitus. Dual-energy X-ray absorptiometry showed relatively increased fat in the head, with decreased fat in the limbs and trunk confirming fat redistribution.
The NGS of LMNA gene showed that the mother and daughter were both heterozygous for a reported c.1444C > T missense mutation[1] which causes the substitution of the Arginine at residue 482 by tryptophan, and was confirmed by Sanger sequencing.
The subjects were started on metformin and insulin therapy was optimized resulting in better glycaemic control.
Conclusions
Clinical suspicion of FPLD2 based on phenotype followed by appropriate biochemical and genetic testing of LMNA gene led to the diagnosis. A clear understanding of the disease process and molecular diagnosis is important to provide appropriate treatment and genetic counseling.
Reference
1. Nabrdalik *et.al*. *Endokrynol Pol* 2013 **64** (4) 306–311.

DOI: 10.1530/endoabs.34.P135

P136

The H-syndrome and next generation sequencing for molecular genetic diagnosis

Nihal Thomas, D M Mahesh, Aaron Chapla, H S Asha, Shrinath Shetty, Sahana Shetty, M Lydia & Renu George
Christian Medical College, Vellore, India.

Background
The H-syndrome is a recently described very rare monogenic systemic autoinflammatory autosomal-recessive genodermatosis with young onset of diabetes without evidence of autoimmunity. It is caused by mutations in the SLC29A3 gene, which encodes the equilibrative nucleoside transporter hENT3.
Objectives
To study the clinical features and confirm the genetic diagnosis in a subject with young onset of diabetes.

Subject and methods
Utilizing next generation sequencing we carried out mutational analysis of SLC29A3 gene in the chr.10q22.1 and selectively investigated this patient for various other features of this genodermatosis.
Case report and discussion
A 20-year-old lady, born to parents of non-consanguineous marriage developed diabetes at the age of 6 years, with ketosis at onset. She required Insulin from the time of diagnosis. Subsequently she noticed progressively increasing hyperpigmented lesions over the trunk and lower limbs and excess hair growth. On examination she had symmetrical large hyperpigmented indurated plaques and hypertrichosis over the lower limbs with sparing of the face. The characteristic skin lesions led to the suspicion of a genodermatosis, namely the H-syndrome and elevated inflammatory markers (CRP and ESR) further added to the clue. The next generation sequencing of SLC29A3 gene was done in our molecular lab, revealed a homozygous mutation c.400C>T, p.R134C, which was confirmed by Sanger sequencing. The term 'H-syndrome' refers to systemic features starting with letter 'H' – hyperpigmentation, hypertrichosis, hyperglycemia, hypogonadism, hypothyroidism, heart anomalies (bicuspid aortic valve), hearing loss, and low height which were present. Clinodactyly, icthyiosis and arthrogryposis of the ankle were peculiar to our subject.
Conclusions
Young onset of diabetes with the dermatological phenotype of pigmentary hypertrichosis, followed by appropriate biochemical and genetic analysis led to the diagnosis of H-syndrome. Genetic testing confirms the diagnosis and would be useful in further counseling.

DOI: 10.1530/endoabs.34.P136

P137

Adrenal incidentalomas, a district hospital perspective

Brian Mtemererwa, Anne de Bray, Anne-Marie O'neill, Alice Webster, Halimah Alazzani, Priyo Sada & Diana Raskauskiene
Walsall General Hospital NHS Trust, Walsall, UK.

An incidentaloma is a mass lesion found by chance on imaging for a reason unrelated to the site of the lesion. Adrenal incidentalomas, as a result of advances in imaging technology, have an increased incidence, especially in the aging population. An endocrine referral is advised to determine if the lesion is functional, to exclude malignancy and offer on going appropriate follow-up.
Aim
To assess the incidence and audit the management of adrenal incidentalomas at a district general hospital.
Method
Abdominal and pelvis CT scan reports between 04/02/2012 and 04/02/2013 were retrospectively reviewed for a comment on adrenal glands. If abnormal, clinical records were reviewed. If not commented on, the scans were reviewed by a consultant radiologist, and clinical records reviewed if the adrenals were abnormal.
Results
2701 images were pooled. 51% (1380) had a comment on adrenals. 45 images had abnormal adrenals of which 32 (2, 4%) were incidentalomas. These incidentalomas were predominantly left sided (20), and four were bilateral. The reported diameters ranged from 9 to 60 mm.
Only three patients had endocrine referrals. Six patients died within 8 weeks of the CT scan due to other co morbidities.49% (1321) of the image reports had no comment regarding the adrenals. Currently 52 of the 1321 scans have been randomly selected and formally reviewed by a radiologist. Five of the 52 had abnormal adrenals, three 'bulky left adrenal glands', and two left sided adenomas, 10 and 15 mm in diameter.
Discussion
Clinicians still need enhanced awareness of the importance to refer patients with adrenal incidentalomas for endocrine evaluation and follow-up. CT scan reporting needs to include a comment on the adrenal glands if imaged, and also prompting clinicians to refer patients with adrenal incidentalomas to the local endocrinologists.

DOI: 10.1530/endoabs.34.P137

P138

Acute confusional state in the thyroid clinic: autoimmunity as a multi-edge knife

Vasileios Chortis[1,2], Matthew Edmunds[2], Edward Littleton[2] & Kristien Boelaert[1,2]
[1]University of Birmingham, Birmingham, UK; [2]University Hopsital Birmingham NHS Foundation Trust, Birmingham, UK.

A 67-year-old lady with a 3-year history of Graves' hyperthyroidism complicated by florid Graves' ophthalmopathy attended the thyroid clinic at University Hospital Birmingham for routine follow up. Her thyrotoxicosis had been managed with long-term carbimazole treatment with good control. Her past medical history included coeliac disease and hypertension. On clinic review, she appeared acutely confused and disorientated and had evidently lost weight. Her family reported a subacute history of cognitive decline over the last month (including disorientation, dyspraxia, and falls), with dramatic deterioration during the preceding few days. She demonstrated coarse tremor of upper and lower limbs but was clinically and biochemically euthyroid. She was admitted to the Clinical Decisions Unit for further investigations and management. A neurological assessment revealed tremor and clonus with hyperreflexia bilaterally. She was started on empirical treatment for meningoencephalitis with antimicrobials and dexamethasone. After two episodes of tonic-clonic seizures, phenytoin was added. She underwent a CT of the brain which was normal, followed by a lumber puncture which excluded an infective cause and was unremarkable aside from a high protein concentration. Her immunology revealed high anti-TPO (1009) and anti-ANA (1:1600) titres. Her EEG revealed diffuse slow-wave activity, while an MRI of the brain was unremarkable. A putative diagnosis of autoimmune encephalitis (likely 'Hashimoto's encephalitis') was made and she responded well to immunosuppression with glucocorticoids (dexamethasone followed by prednisolone). She was discharged on prednisolone (tapering regime) 10 days after her admission. At follow-up a month later she showed complete neurological recovery. Autoimmune encephalitis in the context of Graves' hyperthyroidism is very rare, with only six cases described in the literature. A prompt referral for urgent work-up and appropriate management with immunosuppression is of the essence and generally ensures a favourable outcome.

DOI: 10.1530/endoabs.34.P138

P139

Jumping the gun: an audit of adrenal biopsies in a tertiary referral centre

Vasileios Chortis[1,2], Kassiani Scordilis[2], Wiebke Arlt[1,2] & Rachel Crowley[1,2]
[1]University of Birmingham, Birmingham, UK; [2]University Hospital Birmingham NHS Foundation Trust, Birmingham, UK.

New adrenal lesions discovered during cross-sectional abdominal imaging pose an increasingly common diagnostic challenge; their initial management should focus on the exclusion of malignancy and autonomous hormone excess. The role of adrenal biopsies in this context is limited and pathologists often struggle to differentiate benign from malignant adrenal tissue even when analysing the entire tumour specimen. Guidelines recommend that adrenal biopsy should only be considered if two criteria are met: firstly, history of extra-adrenal malignancy and an isolated new adrenal lesion with suspicious imaging criteria; secondly, biochemical exclusion of phaeochromocytoma. We reviewed all cases of adrenal biopsies performed at University Hospital Birmingham (UHB) in the time period from January 2004 to October 2013, with a view to assessing compliance with standards of best practice, reviewing histopathology and clinical medical records. We identified 18 UHB patients with adrenal biopsies in the defined 10-year period. The decision for adrenal biopsy was taken in accordance with guideline recommendations in only three patients; an additional 12 fulfilled the clinical criterion only and in the remaining three none of the required criteria was fulfilled. MDT review prompted biopsy in seven of 18 but only three patients underwent endocrine review. In ten of the 14 patients with underlying history of malignancy the histopathology result was informative, prompting chemotherapy in six of them. All but two patients died within a year of biopsy (median 2 months, range 3 days–12 months). In one patient with a recent history of melanoma the biopsy indicated adrenocortical adenoma; the adrenal mass was removed surgically 3 years later due to significant growth and histopathology revealed a melanoma metastasis embedded in a large phaeochromocytoma. In conclusion, patients undergoing adrenal biopsies are frequently managed hazardously, with only the minority receiving MDT and endocrine review. Histopathology is regularly non-informative and can be misleading.

DOI: 10.1530/endoabs.34.P139

P140

Is levothyroxine availability enhanced by grapes?

Ishrat Khan, Andrew Aldridge, Ruhina Ahmed & O E Onyebuchi
Prince Charles Hospital, Merthyr Tydfil, CF47 9DT Wales, UK.

Introduction
Patients with hypothyroidism are routinely advised to take levothyroxine without food or beverages but the pharmacokinetic effect of many common foods are unknown. We describe a patient with an unusually high levothyroxine requirement who only achieved adequate replacement by taking grapes along with levothyroxine. Through a cycle of challenge, withdrawal, and-re-challenge, we were able to demonstrate that levothyroxine availability was enhanced by grapes in this case.
Case report
A 30-year-old lady underwent total thyroidectomy for papillary thyroid cancer which had developed on a background of long-standing Hashimoto's thyroiditis. Prior to this a stable euthyroid state had been maintained for many years with 100 μg daily of levothyroxine which she regularly took with grapes to make the tablets palatable. Following surgery she continued taking levothyroxine but was advised to avoid taking grapes with the tablets. Her thyroid status subsequently deteriorated and TSH remained persistently elevated even with levothyroxine doses as high as 350 μg daily. Other factors such as poor compliance, concomitant medication use, and co-morbid conditions like coeliac disease, malabsorption, and pernicious anaemia were excluded. Resumption of grapes led to a remarkable reduction of levothyroxine requirements to 125 μg daily. Three months afterwards she stopped taking grapes during a 3 weeks holiday abroad but continued to take levothyroxine on an empty stomach. Thyroid function a week after she returned showed that she had become hypothyroid again. Re-introduction of grapes with levothyroxine once more restored normal thyroid status.
Conclusion
To the best of our knowledge this is the first report of high levothyroxine requirements corrected by ingestion of grapes. The mechanism of this effect is unclear but it is plausible that the grapes provide a favourable acidic environment for levothyroxine absorption. Further studies will be required to confirm this effect and elucidate the underlying pharmacokinetic mechanisms.

DOI: 10.1530/endoabs.34.P140

P141

Granulomatosis with polyangiitis presenting as a pituitary lesion

Louise Christou[1], Mahesh Katreddy[2], Shouma Dutta[2], Sanjeet Kamath[2], Simon Shaw[2] & Biju Jose[2]
[1]School Of Medicine, Keele University, Stoke-on-Trent, UK; [2]University Hospital of North Staffordshire, Stoke-on-Trent, UK.

Granulomatosis with polyangiitis (GPA), previously known as Wegener's granulomatosis, is a rare heterogeneous vasculitic disease characterised by necrotising granulomatous inflammation typically affecting lungs and kidneys. GPA affecting pituitary is rare and usually results in posterior pituitary involvement causing diabetes insipidus. We describe a case of GPA, presenting with pituitary involvement causing headaches and anterior pituitary hormone deficiencies.
Middle-aged lady presented with 7 weeks of worsening retro-orbital headaches. Initial investigations included MRI head which showed a sellar lesion, suggestive of inflammatory conditions like sarcoid or metastasis. She had no features of pituitary hormone deficiencies, systemic vasculitides, or other system involvement. Tests confirmed hypopituitarism involving gonadal and thyroid axes. Empirical treatment with intravenous methylprednisolone, for possible sarcoid, improved symptoms. A staging CT scan showed localised pulmonary consolidation. She was discharged on hydrocortisone and thyroxine replacement with plans for further assessment. She re-presented 2 weeks later with dyspnoea; the pulmonary lesion had significantly enlarged. A diagnostic lobectomy revealed extensive suppurative necrotising granulomatosis on histology. Vasculitic screen revealed anti-neutrophil cytoplasmic antibody proteinase 3 (cANCA-PR3), consistent with GPA. Serum ACE was normal. Pulse cyclophosphamide therapy and maintenance with azathioprine/prednisolone resulted in clinical improvement. A repeat pituitary MRI scan, seven months later, showed shrinkage of the pituitary lesion.
The pathophysiology of pituitary GPA is described as granulomatous invasion from nasal or paranasal lesions, *in-situ* granuloma formation, and/or inflammation of the pituitary vasculature. This case is unusual in its involvement of pituitary gland in the initial presentation and absence of the usually described diabetes insipidus. The case highlights the importance of assessing the pituitary in granulomatous diseases, especially GPA. Early identification of pituitary GPA and replacement of deficient pituitary hormones is crucial in the often catastrophic disease course. Appropriate Immunomodulatory therapy can prevent the otherwise inevitable destruction of the pituitary gland.

DOI: 10.1530/endoabs.34.P141

P142

A case of hypoadrenalism

Funke Akiboye, Adam Nicholls & Triston Richardson
Royal Bournemouth Hospital, Bournemouth, UK.

A 69-year-old man was referred to Endocrine clinic after presenting to his GP with dizziness, syncope, and fatigue. 0900 h cortisol was low at 39 nmol/l. The remainder of the anterior pituitary profile was unremarkable. He had been diagnosed with HIV in 1997 and was treated with highly active anti-retroviral therapy (truvada 1 OD, ritonavir 100 mg OD, and darunavir 800 mg OD) in addition to acyclovir 400 mg OD with control of his CD4 count and viral load. In addition, treating unexplained weight loss and intermittent anorexia he had been commenced on megesterol acetate 160 mg in 2011.
Hydrocortisone was started at a dose of 10/5/5 mg with resolution of his symptoms.
He was advised to stop megesterol, and was subsequently weaned off hydrocortisone. Repeat early morning cortisol was 201 nmol/l in November 2013 with no ongoing symptoms of hypoadrenalism. Short Synacthen testing was normal.
Owing to the cytochrome P450 enzyme inhibiting effect of some of the ritonavir-based anti-retrovirals, Cushing's and hypoadrenalism have been noted in the use and withdrawal respectively of even inhaled steroids in HIV. Megesterol has some glucocorticoid action and there have been reports of secondary adrenal suppression in patients with disseminated malignancy and AIDS.
Hypoadrenalism has been noted after withdrawal of megesterol after prolonged use of years and assessing for this is advised on stopping protracted courses. This case demonstrates profound symptomatic adrenal suppression induced by lower doses of megesterol used only intermittently in conjunction with ritonavir. Owing to the potent enzyme inhibiting effect of ritonavir the possibility of hypoadreanlism should be kept in mind in patients stopping megesterol even with intermittent use at low doses.

DOI: 10.1530/endoabs.34.P142

P143

Mayer-Rokitansky-Kuster-Hauser syndrome and pituitary adenoma: a co-incidence?

Rajendran Bellan Kannan, Ananth Nayak, Laks Varadhan,
George Varughese & Biju Jose
University Hospital of North Staffordshire, Stoke-on-Trent, UK.

Background
Mullerian agenesis or Mayer-Rokitansky-Kuster-Hauser (MRKH) syndrome is a congenital failure of the Mullerian duct to develop, resulting in complete or partial absence of the cervix, uterus, and vagina. It can be isolated (MRKH type I) or associated with renal, vertebral, and, to a lesser extent, auditory and cardiac defects (MRKH type II). Pituitary disease is not a known association. We report a patient who has isolated MRKH syndrome (MRKH type I) and a pituitary adenoma, diagnosed concomitantly.
Case
Young female was referred with primary amenorrhoea. On assessment she had normal secondary female sexual characteristics. There was no significant family history. Investigations were organised to rule out structural or genetic abnormalities and hypothalamic causes in view of low BMI (19). Oestradiol was low, 70 (93–1400 pmol/l) with inappropriately low–normal gonadotrophins. Other pituitary hormone axes were normal. MRI of pituitary showed 6mm pituitary adenoma. MRI pelvis showed absent uterus/cervix with severely hypoplastic vagina, confirming Mullerian hypoplasia. Cytogenetics showed 46XX. Her renal ultrasound was normal. The gonadotrophins and oestradiol normalised spontaneously, presumably with weight gain. Moreover, she had features of adequate oestrogenisation with normal secondary sexual characters. Future fertility and surrogacy options were discussed in a gynaecology clinic setting, in addition to providing details of support groups. The pituitary adenoma is under review from the endocrine clinic.
Discussion
The estimated prevalence of MRKH syndrome is one in 4500 female births. The etiology of MRKH syndrome remains unclear. The patients often require considerable psychological support following diagnosis. There is no known

association with pituitary disease. To the best of our knowledge this is the first case of co-existing MRKH syndrome and pituitary adenoma from reported from the Western world.

DOI: 10.1530/endoabs.34.P143

P144

Madness on the medical ward: ectopic ACTH producing tumour

Amar Puttanna[1], Viraj Patel[1], Diana Raskauskiene[1] & Richard Clayton[2]
[1]Walsall Hospitals NHS Healthcare Trust, Walsall, UK; [2]University Hospital of North Staffordshire NHS Trust, Stoke-on-Trent, UK.

A 51-year-old Afro-Caribbean lady with a history of primary hyperparathyroidism, type 2 diabetes and hypertension was admitted with confusion. Her medication at the time included five anti-hypertensives.

She was treated for an infection but despite a long course of antibiotics she remained confused with visual hallucinations and aggression.

She was noted to have resistant hypertension with systolic blood pressures over 220 mmHg and persistent hypokalaemia, this prompted assessment for endocrine causes. On examination there was no evidence of cushingoid features. Low dose dexamethasone suppression tests revealed morning cortisols between 500 and 600 nmol/l however due to suspected non-concordance from the psychosis a repeat suppression test with 2 mg i.v. dexamethasone was administered. This also showed failure to suppress with a morning cortisol of 875 nmol/l and ACTH of 149 ng/l.

Baseline cortisol was 972 nmol/l and ACTH was 250.9 ng/l; following a high dose dexamethasone suppression test, cortisol was 885 nmol/l with ACTH 247 ng/l confirming ACTH dependant Cushing's syndrome.

MRI with contrast showed no evidence of pituitary adenoma and a CT scan revealed a 3.3 cm right lung nodule with bilateral adrenal hyperplasia compared to previous scans.

Her psychosis worsened despite antipsychotic medication therefore etomidate 3 mg/kg was administered in an ITU setting but was ineffective. Subsequently ketoconazole 600 mg TDS was tried with no improvement and random plasma cortisols remained over 1000 nmol/l.

She underwent a resection of the nodule which stained positive for TTF-1, synaptophysin, CD56 and chromogranin, in keeping with carcinoid. The patient's psychosis, blood pressure and glycaemia improved with cortisol levels settling to 100 nmol/l.

This complex case highlights the unusual presentation of Cushing's syndrome from an ACTH secreting carcinoid lung tumour. It highlights the extreme and resistant psychosis that can accompany such patients, not previously described in literature. Acute psychosis in a young patient with resistant hypertension, diabetes and hypokalaemia should lead to suspicion of hypercortisolaemia.

DOI: 10.1530/endoabs.34.P144

P145

Training needs in adolescent health and transition in paediatric and adult trainees in diabetes and endocrinology in the UK

Helena Gleeson[1], Rohana Wright[2], Rachel Besser[3], Kelly Cheer[4], Paul Dimitri[5], Simon Chapman[6] & on behalf of AYASIG[1]
[1]Leicester Royal Infirmary, Leicester, UK; [2]Royal Victoria Infirmary, Newcastle, UK; [3]Great Ormond Street Hospital, London, UK; [4]Manchester Royal Infirmary, Manchester, UK; [5]Sheffield Children's Hospital, Sheffield, UK; [6]King's College Hospital, London, UK.

Background

Training is adolescent health and transition is lacking in medical education. Clinicians working in diabetes and endocrinology across paediatric and adult services need to have the skills to work effectively with this age group. Our aim was to identify the current state of training and to ascertain training needs in trainees.

Methods

A questionnaire was developed based on existing questionnaires. Using trainee representatives all paediatric and adult trainees in diabetes and endocrinology were emailed a link to the online questionnaire.

Results

Of the 85 responses, 51 were from adult trainees and 34 were from paediatric trainees (nine grid). All grades were represented and all parts of the UK. Around 60% of all trainees felt their training in and clinic exposure to adolescent health and transition was minimal or non existent. This was more marked within endocrinology (74%) than in diabetes (39%). Barriers to providing developmentally appropriate care included lack of clinic time (73%) and lack of training (70%). Training needs in adolescent health and transition were higher in endocrinology (91%) than in diabetes (76%). In excess of 50% had received training on growth and puberty and diabetes. Training was at $<25\%$ in a number of areas including benefit entitlement, independent living, vocational and educational and psychosocial issues. The highest training needs, with more than 50% rating their needs four or five (out of five), were identified in available resources, interagency working, endocrinology, and transition. More paediatric trainees had received training than adult trainees in the majority of areas. Despite this paediatric trainees rated their needs as higher than adult trainees.

Conclusion

This questionnaire has highlighted the need to improve training in adolescent health and transition for paediatric and adult trainees in diabetes and endocrinology. Paediatric trainees receive more training and have a higher awareness of a need for training.

DOI: 10.1530/endoabs.34.P145

P146

Delayed hypocalcaemia following treatment of malignant hypercalcaemia with denosumab

Amar Puttanna, Ahsan Khan & Noushad Padinjakara
Walsall Hospitals NHS Healthcare Trust, Walsall, UK.

Denosumab is a MAB currently licensed for use in treatment of osteoporosis. It is designed to inhibit RANK ligand to prevent bone breakdown by osteoclasts. Its use for treatment of malignant hypercalcaemia is still to be fully investigated.

We present the case of a 71-year-old south Asian gentleman with recurrent resistant hypercalcaemia secondary to follicular non-Hodgkin's lymphoma. Calcitriol levels were elevated which was thought to be lymphoma related. Despite numerous administrations of bisphosphonates including pamidronate and monthly zolendronate, the patient's calcium levels remained elevated around 3.11 mmol/l even reaching 4.6 mmol/l.

Owing to significant elevation of calcium and resistance to conventional methods (including dexamethasone), denosumab 60 mg s.c. was administered as a means of controlling the hypercalcaemia. The patient's calcium remained elevated on discharge and up to 1 month later. Approximately 1 month following administration the patient's calcium levels were recorded within the normal range (2.13 mmol/l) however it began to decrease further over the course of the next few weeks and the patient became significantly hypocalcaemic (1.58 mmol/l) requiring calcium supplementation.

There is currently limited literature on the use of denosumab in managing hypercalcaemia in malignancy and it is only licensed for preventing skeletal related events in bone metastases from solid tumours and myeloma. There is no literature on its use in lymphoma. This case highlights the difficulties in managing malignant hypercalcaemia and offers an effective new therapeutic option in the form of denosumab. It also highlights the importance of regular follow up to monitor calcium levels as there is the possibility of a delayed effect of denosumab in treating hypercalcaemia.

This is the first documented case of denosumab use in hypercalcaemia secondary to lymphoma. It also provides evidence of delayed hypocalcaemia as a side effect of denosumab therapy up to 1 month following administration.

DOI: 10.1530/endoabs.34.P146

P147

Non islet cell tumour hypoglycaemia: management strategies for a rare and challenging condition

Dhanya Kalathil, Simon Roughneen, Momin Shah, Ali Akram, Reza Zaidi, Tejpal Purewal, Aftab Ahmad & Philip Weston
Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, Merseyside, UK.

We previously reported a case of non islet cell tumour hypoglycaemia (NICTH) due to increased 'big'-IGF2 production in a lady with haemangiopericytoma (HAP). We now describe the challenges in managing this lady who had recurrent, disabling hypoglycaemia requiring frequent hospitalisation.

This patient presented with symptomatic hypoglycaemia 15 years after the diagnosis of parasaggital HAP. At presentation she had tumour recurrence with liver and brain metastases.

Surgical resection was not an option due to extent of disease, but she received chemotherapy to reduce tumour bulk. When this failed to alleviate symptoms, we commenced prednisolone 10 mg OD to which she responded well for a few months. Her hypoglycaemia returned, prompting addition of recombinant GH (rGH) to her therapy. We limited the use of glucagon for treatment of hypoglycaemic emergencies.
Despite combination therapy hypoglycaemia relapsed, and hepatic artery embolization was undertaken to decrease tumour bulk. Diazoxide was considered, but was abandoned due to poor tolerance. She had s.c. somatotropin briefly, which was stopped when octreotide scanning showed no tracer uptake.
As her condition deteriorated, debulking surgery or localised radiotherapy for liver metastases became impossible. Hypoglycaemia in the last days of her life was managed with i.v. dextrose.
Hypoglycaemia due to NICHT is difficult to treat. Complete resection of the tumour cures hypoglycaemia, but when impossible, reduction of tumour bulk will help. Glucocorticoids and rGH form the mainstay of medical therapy. Glucocorticoids provide symptom relief by stimulating gluconeogenesis. How rGH works is not understood, but it can be used alone or in combination with steroids.
This case is interesting as much for the challenges of management as for the rarity of the diagnosis.

DOI: 10.1530/endoabs.34.P147

P148

Diabetes: the forgotten complication of parathyroidectomy

Nantia Othonos[1], Vinod Patel[1,2], Rajiv Nair[1], Stephen Ayre[3] & Ponnusamy Saravanan[1,2]
[1]Academic Department of Diabetes, Endocrinology and Metabolism, George Eliot Hospital, Nuneaton, UK; [2]Warwick Medical School, University of Warwick, Coventry, UK; [3]William Harvey Library, George Eliot Hospital, Nuneaton, UK.

A 64-year-old female presented to the acute medical take with hypercalcaemia. She had a 3 weeks history of polyuria, polydipsia, constipation, intermittent abdominal pain and feeling depressed. She reported weight loss of 3.5 kg over the past 6 months. She denied symptoms of dysphagia, dyspnoea, haemoptysis, haematemesis, or malaena. Her past medical history included anaemia, ischaemic heart disease, chronic obstructive pulmonary disease and uterine prolapse. She was an ex-smoker with 45 pack-year history. She drank ten units of alcohol. Investigations revealed: corrected calcium, 3.35 mmol/l; PTH, 28.9 pmol/l; vitamin D, 39.4 μg/l, and normal glucose and U&Es. Parathyroid sestamibi scan showed focal uptake in the inferior left thyroid lobe. Initial diagnosis of Primary hyperparathyroidism was made but the parathyroidectomy showed a likely parathyroid carcinoma but without all features of cancer. Two months later, her symptoms of polyuria and polydipsia returned but had normal calcium and PTH levels. However, her blood glucose level was 49.1 mmol/l (HbA1c, 145 mmol/mol). Her BMI was 23. She was started on basal bolus insulin regime. Four months later, her insulin requirements decreased dramatically (Novorapid two units BD and Glargine four units; HbA1c, 46 mmol/mol) and was stopped. Ten months later she was still off insulin with a HbA1c of 43 mmol/mol.
It is known that both high PTH and hypercalcaemia can increase insulin resistance resulting in compensatory hyperinsulinaemia. While transient reduction in insulin secretion has been demonstrated in post-parathyroidectomy patients, onset of insulin requiring diabetes has never been reported in the literature. Our patient clearly demonstrates the relative insulin deficiency following parathyroidectomy resulting in diabetes. It highlights the need for close monitoring of glucose level in post-parathyroidectomy patients, especially if they had prolonged high calcium and PTH levels prior to surgery.

DOI: 10.1530/endoabs.34.P148

P149

Adrenocortical carcinoma, where's the delay?

Aftab Aziz[1], Rob Dyer[2], Julie Dunn[1], Andy Goodman[1] & Antonia Brooke[1]
[1]Macloed Diabetes and Endocrine Centre, Royal Devon and Exeter Hospital, Exeter, UK; [2]Department of Diabetes and Endocrinology, Torbay Hospital, Torquay, UK.

Adrenocortical carcinomas are rare malignancies (incidence 1–2/one million). They present in a variety of ways and carry a poor prognosis. Prompt diagnosis and early intervention offers better outcome.
We describe 5 cases of adrenocortical carcinoma to help identify and improve delays in their pathway of care. Five patients (four females and one male), age range 38–76 years, presented between September 2011 and April 2013. Three patients referred by GP had suggestive symptoms for previous 3–6 months, with a delay in diagnosis. Three presented with mass symptoms and two with secretory symptoms. All tumors were stages III–IV at diagnosis (adrenal size ranged from 8 to 20 cm). First MDT waiting time was $<$ 1 week to 2 months (post-operatively). The patient who was unsuitable for resection had a decision about complex surgery 10 days after referral to tertiary MDT – an anguished wait as an inpatient. A further patient waited 3 months for a joint procedure at a tertiary cancer centre. All patients had Mitotane prescribed immediately after histological confirmation and presence of metastases, except one patient this was delayed until she presented 2 months later with progressive disease. Only one patient managed therapeutic levels (due to side effects). All patients with progressive disease were offered chemotherapy and were either too unwell (two patients) or declined (two patients).
Patients present late with adrenal cancer. The longest delay was in initial diagnosis due to the rarity and complexity of symptoms not being recognised in primary care. However there were other delays in biochemical work up, discussion through MDT, surgical treatment and post-operative mitotane initiation. All patients had care involving more than one centre, from specialist centres to district general hospitals. Logistics of diagnosis, investigation and treatment across primary, secondary and tertiary centres can be improved for optimal care.

DOI: 10.1530/endoabs.34.P149

P150

Tertiary hyperparathyroidism: a long-term complication of pseudohypoparathyroidism type 1b?

Justyna Witczak, Penelope Owen & Mike Page
Royal Glamorgan Hospital, Pontyclun, UK.

Introduction
Pseudohypoparathyroidism is a rare group of heterogenous disorders. PHP1b consists of renal resistance to PTH in the absence of other physical or endocrine abnormalities and is associated with reduced 1,25-OH vitamin D synthesis, increased phosphate secretion and hypocalcaemia. Despite calcium and vitamin D replacement many patients still have chronically elevated PTH.
Case description
A 31-year-old white female presented with symptoms of tetany with her bloods revealing hypocalcaemia of 2.05 mmol/l, increased ALP (263 IU/l) and increased PTH (1100 ng/l). She had normal 25-hydroxycholecalciferol. The hand radiograph suggested osteitis fibrosa. Secondary hyperparathyroidism was excluded and diagnosis of pseudohypoparathyroidism was made and patient was commenced on vit D2 and calcium supplements. 25 years later she presented with hypercalcaemia, raised PTH and borderline low vit D levels and was switched from calcitriol to α-calcidiol which she stopped as she couldn't tolerate the preparation. Her calcium levels remained elevated, vit D became undetectable and PTH soon exceeded 150 pmol/l. The DEXA scan showed L1–L4 osteopenia and US KUB-marked bilateral cortical thinning. US neck suggested left lower parathyroid adenoma confirmed by a Sestamibi scan which revealed a well defined 2$\times$1 cm left inferior parathyroid adenoma and possibly a second intrathyroid 8 mm parathyroid adenoma. Patient was eventually established on 2200 units of cholecalciferol OD and weekly alendronate with normalisation of calcium and vitamin D levels and gradual decline in PTH levels. Decision was made not to proceed with parathyroidectomy as it could potentially lead to hyperplasia of another parathyroid gland.
Conclusion
The management goal in PHP1b is to achieve normacalcaemia and maintain PTH as low as possible. However, PHP1b patients are at risk of developing tertiary hyperparathyroidism.

DOI: 10.1530/endoabs.34.P150

P151

Liraglutide as an effective therapeutic agent in a patient with Prader Willi syndrome and type 2 diabetes

Justyna Witczak & Penelope Owen
Royal Glamorgan Hospital, Rhondda Cynon Taff, UK.

Introduction
Prader Willi syndrome is a genetic disorder associated with learning disability, hyperphagia and obesity which leads to early development of obesity related complications. The weight and appetite management remains a challenge with not much success with appetite suppressant drugs and bariatric surgery.
GLP-1 receptor analogues appear to be a promising alternative given their effect on appetite control, weight loss and HbA1c and were shown to decrease the levels of plasma ghrelin in PWS patients.
Case history
A 25-year-old white female with PWS presented to diabetic clinic with newly diagnosed type 2 diabetes. She was delivered at 35 weeks gestation (weight 2.63 kg) but at 4 weeks became unwell and hypotonic which raised the clinical suspicion of PWS. The subsequent molecular analysis revealed kariotype 46, XX, del(15) (q11.2q11.2).
At the time of clinical manifestation of her diabetes she already had an extensive list of obesity-related comorbidities: lymphoedema, previous PE, gout and previous cholecystectomy. She struggled to manage her weight with dietary interventions.
She was initially treated with metformin and gliclazide which failed to achieve the target HbA1c nor improved her weight. A decision was made to give her a trial of liraglutide – 0.6 mg daily subsequently titrated to 1.2 mg daily with metformin 500 mg bd being continued.
Results
In October 2011, when liraglutide was commenced patient's weight was 148 kg (BMI 65) with HbA1c of 64 mmol/mol. By May 2012 the weight has improved to 145 kg and HbA1c to 51 mmol/mol. One year later her weight has further improved to 139 kg (BMI 61) and her glycaemic control remains within target (54 mmol/mol).
Conclusions
Although there are only few cases reported on GLP1-RA in PWS patients the results are encouraging given the potential possibility of extending the use of these agents for obesity management in future.

DOI: 10.1530/endoabs.34.P151

P152

Cushing's disease with hepatic adrenal rests

Daniel Border[1,2], Saboor Aftab[1,2], Lavanya Pelluri[1], Thomas Barber[1,2] & Harpal Randeva[1,2]
[1]University Hospitals Coventry and Warwickshire (UHCW), Coventry, UK; [2]Warwick Medical School (WMS), University of Warwick, Coventry, UK.

We present the case of a 58-year-old woman who presented 30 years ago. She was noted to have abdominal obesity, moon-face and skin bruising/thinning, as well as hypertension. Serum cortisol was elevated and she was presumed to have Cushing's disease (although we have no record of dynamic tests or imaging from the time of her original presentation). Owing to her desire for future fertility, she underwent bilateral adrenalectomy at the age of 31 years. She received hydrocortisone replacement therapy and was lost to follow-up.
She re-presented 15 years post-surgery having developed type 2 diabetes mellitus and osteoporosis. At this time, her serum cortisol and 24-h urinary cortisol were markedly elevated (803 nmol/l and 3022 nmol respectively) with an elevated serum ACTH 277 pg/ml suggestive of recurrence. At the time, both MRI pituitary and CT scan of her chest and abdomen were reported as normal (with prior bilateral adrenalectomy noted). Results from HDDST and CRH tests were consistent with Cushing's disease. An iodocholesterol^{131} radio-labelled scan showed presence of adrenal rest tissue within the liver. IPSS confirmed pituitary-origin for raised ACTH and she was commenced on ketoconazole. At the age of 54 years, 23 years following her original surgery, she was demonstrated on pituitary MRI to have developed a microadenoma. She underwent subsequent trans-sphenoidal resection. Although histology failed to show presence of any corticotrophinoma, the patient has remained in biochemical remission from her Cushing's disease.
This case raises some important learning points:
i) The need for careful investigation of Cushing's and a targeted approach to management;
ii) the importance of clinical and biochemical facts when faced with an inconsistent histopathology report; and
iii) although, she does not fulfil criteria for Nelson's syndrome, the importance of close follow-up of patients with Cushing's disease is demonstrated.

DOI: 10.1530/endoabs.34.P152

P153

The prevalence and mortality in hospitalised patients with mild, moderate, and severe hyponatremia

Jayadave Shakher, Nirav Gandhi, Lisa Shepherd, Christopher Pitt & Kamaljit Chatha
Heart of England NHS Trust, Birmingham, West Midlands, UK.

Introduction
Hyonatraemia, defined as serum sodium <135 mmol/l is the commonest electrolyte abnormalities and is associated with increased morbidity and mortality.
Aim
To determine the prevalence of mild, moderate, and severe Hyponatraemia in the hospitalised patients and estimate mortality between these three groups and compared to Normonatraemia.
Methods
This is a retrospective audit in patients admitted to hospital from July 2010 to July 2011. The data are obtained from hospital patients' Registry. The admission sodium is used for statistical comparisons. The hospital governance approved the audit. Normonatraemia is defined as serum sodium 135–144 mmol/l, hyponatraemia <135 mmol/l, and hypernatraemic >145 mmol/l.
Results
The study population was 98 078. The mean age was 55.85 ($\pm$S.D. 20.67) and 55.12% males and 44.88% females. 45.26% were over 60 years. Hyponatraemia was observed in 5.05%. There were significant age difference between the different sodium groups with normal range being the youngest and those with severe hyponatraemia, the oldest with men in severe hyponatraemia category ($P<0.001$) There were significant differences in mortality with lowest mortality in normal group and the highest death rate in hypernatraemic group ($P<0.001$).
Conclusion
Hyponatraemia as well as hypernatraemia is associated with increased mortality.

Mortality	Sodium range (mmol/l)				Sex	Freq.	Percent
	<135	135–144	≥145	Total			
30 days					F	54 064	55.12
No	4238	86 805	3002	94 045	M	44 014	44.88
Yes	716	2419	898	4033	Total	98 078	100.00
Total	4954	89 224	3900	98 078			
1 year							
No	3773	83 714	2848	90 335			
Yes	1181	5510	1052	7743			
Total	4954	89 224	3900	98 078			
2 years							
No	3485	81 260	2 751	87 496			
Yes	1469	7964	1149	10 582			
Summary of age							
Sodium (mmol/l)	Mean	S.D.	Freq.				
<135	67.920065	18.599677	4954				
135–144	54.797039	20.515345	89 224				
≥145	64.819487	19.840263	3900				
Total	55.858429	20.677769	98 078				

DOI: 10.1530/endoabs.34.P153

P154

Profound hyponatraemia secondary to indapamide

Julia Prague & Ben Zalin
Department of Endocrinology, Hertfordshire, UK.

Background
Severe hyponatraemia and other electrolyte disturbances secondary to indapamide are documented in the literature. We report two cases that presented with neurological sequelae of profound hyponatraemia without seizures secondary to indapamide.
Cases
An 86-year-old female presented with a history of confusion, vomiting and frontal headache associated with lethargy and anorexia. She had been started on

indapamide 1 month earlier. She was on no other medications. Her abbreviated mental test score was 2/10. She was euvolaemic and had left basal crepitations. Serum sodium was 99 mmol/l, potassium 3.5 mmol/l, and magnesium 0.58 mmol/l. Chest X-ray was consistent with infection. The patient was managed in a high dependency environment and the sodium steadily increased with slow N.Saline and cessation of the drug.
A 68-year-old male was brought to hospital by his wife because of confusion. He had been started on perindopril 1 month earlier and indapamide 3 months prior. He had presented to his GP 2 weeks before complaining of a 'muzzy head'. Serum sodium was 100 mmol/l and potassium was 2.6 mmol/l. The patient was managed as above.
Conclusions
These cases highlight the profound hyponatraemia that can result from indapamide therapy. As the sodium loss is progressive, the hyponatraemia is chronic and therefore well tolerated. Any neurological symptoms in patients on diuretics or anti-hypertensives should have an urgent blood test to measure electrolytes. Patients with neurological sequelae secondary to hyponatraemia should be managed in a high dependency environment irrespective of serum sodium concentration.

DOI: 10.1530/endoabs.34.P154

Cytokines and growth factors

P155

Modelling hepatic encephalopathy *in vitro*: molecular and functional consequences of hyperammonaemia in C6 glioma cells

Samantha Mirczuk[1], Camille Robson[1,2], Emily Stacey[1,2], Robert Fowkes[1] & Victoria Lipscomb[2]
[1]CBS, Royal Veterinary College, London, UK; [2]CSS, Royal Veterinary College, Hatfield, UK.

Hepatic encephalopathy (HE), a syndrome of neurological abnormalities caused by impaired liver function, is induced by many chronic liver diseases, such as liver failure or hepatic portosystemic shunts. Whilst multiple substances reach toxic levels in the systemic circulation of HE patients, hyperammonemia (excessive ammonia) is known to be a major contributing factor to the neurological disturbances. Astrocytes utilise ammonia in generating glutamine leading to brain oedema and neurocytotoxicity in HE patients, through unknown mechanisms. Interestingly, astrocytes are known to be targets for C-type natriuretic peptide (CNP) action. In this study, we examine molecular changes occurring in astrocytes during hyperammonaemic conditions. Rat C6 glioma cells were treated with 0 – 10 mM NH_4Cl (an ammonia donor) or 100 nM CNP for up to 72 h before extracting total RNA, and utilising multiplex quantitative RT-PCR to examine gene expression (*Gad1, Gfap, S100b, Hmox1, Tspo, Insr, Npr1, Npr2, Npr3, Nppa, Nppb, Nppc*, simultaneously). After 72 h, hyperammonaemia caused a significant increase in *Insr* ($^{**}P<0.01$), *Npr3* ($^{*}P<0.05$), and *Nppb* ($^{***}P<0.001$) expression, but inhibited *Gfap* ($^{*}P<0.05$). In contrast, the effects of CNP were more rapid, with significant increases in *S100b* ($^{*}P<0.05$), *Hmox1* ($^{*}P<0.05$), *Tspo* ($^{*}P<0.05$), and *Npr2* ($^{*}P<0.05$) expression within 24 h. To establish whether hyperammonaemia affects CNP function in astrocytes, C6 cells were pre-treated with 10 mM NH4Cl for 24 or 1 h, prior to stimulation with 100 nM CNP for 0, 5 or 15 min. CNP-stimulated cGMP accumulation underwent homologous downregulation between 5 and 15 mins, regardless of exposure to hyperammonaemia. However, acute (1 h) but not chronic (24 h) induction of hyperammonaemia caused inhibition of cGMP accumulation consistent with heterologous desensitisation. Collectively, these data identify novel molecular targets that may contribute the HE phenotype in astrocytes, and suggest an interaction between ammonia and CNP signalling.

DOI: 10.1530/endoabs.34.P155

P156

Spatial expression profiling reveals tissue-specific sexual dimorphism of the natriuretic peptide system in mice

Samantha Mirczuk[1], Camille Robson[1], Benjamin Robinson[1], Andrew Lessey[1], Victoria Lipscomb[2], Imelda McGonnell[1] & Robert Fowkes[1]
[1]CBS, Royal Veterinary College, London; UK, [2]CSS, Royal Veterinary College, Hatfield, UK.

The mammalian natriuretic peptide family consists of three members, Atrial-, B-type, and C-type natriuretic peptides (ANP, BNP, and CNP respectively), and exert their effects on blood pressure, sodium homeostasis, growth and reproduction, via their selective guanylyl cyclase receptors, GC-A or GC-B. Previous attempts to map spatial expression profiling have generated conflicting results, due predominantly to the range of techniques employed, and the relative paucity of tools to examine the natriuretic peptide system at the molecular level. Here, we describe a comprehensive spatial expression screen of the natriuretic peptide system in mouse tissues, using our novel multiplex qRT-PCR system. Total RNA was extracted from adipose, adrenal, brain, heart, kidney, liver, ovary, and testis of 12-week-old C57/B6 mice, prior to multiplex qRT-PCR analyses (for *Nppa*, *Nppb*, *Nppc*, *Npr1*, *Npr2*, *Npr3*, *Corin*, *Furin*, *Actb*, *Gapdh*, and *Rpl19*). Spatial expression profiles for *Nppa* (ANP) and *Nppb* (BNP) were tightly conserved, with high levels of each transcript detected in cardiac ventricles. *Nppc* (CNP) expression was more broadly distributed, with the highest expression levels seen in forebrain. Expression of *Npr1* and *Npr2* was consistently high in all tissues examined, although the abundance of *Npr1* expression in forebrain was only half that seen in other tissues. In contrast, *Npr3* (clearance receptor), *Corin*, and *Furin* (processing enzymes) were ubiquitously expressed. On further examination, sexually dimorphic differences were seen for *Nppc* (adrenal and adipose), *Npr3* (kidney), *Furin* (cardiac ventricles), and *Corin* (liver), suggesting gender-specific roles for these components of the natriuretic peptide system in these tissues. These data represent the most comprehensive tissue-specific expression profiling of the natriuretic peptide system in mice, suggest potential gender-specific roles for CNP signalling in adrenal and adipose tissue, and confirm that the CNP/GC-B is the predominate natriuretic peptide system in the brain.

DOI: 10.1530/endoabs.34.P156

P157

The remote organ effects of acute kidney injury in a porcine model

Louise Dunford[1,3], David Gardner[2], Benjamin Duckworth[2], Saoirse O'Sullivan[2] & Mark Devonald[2,3]
[1]De Montfort University, Leicester, UK; [2]University of Nottingham, Nottingham, UK; [3]Nottingham University Hospitals Trust, Nottingham, UK.

Background
Acute kidney injury (AKI) is common and serious, with no specific treatment. AKI may increase the risk of dysfunction in organs other than the kidney, further increasing the morbidity associated with AKI. The mechanisms of organ cross-talk after AKI are unclear. Small animal models have suggested that infiltration of inflammatory cytokines into extra-renal organs leads to cellular extravasation, oedema and further injury. In this study we used a large animal model to investigate the remote organ effects of AKI.
Design
We used an established porcine model of warm ischaemia–reperfusion induced AKI (control, $n=12$ and AKI, $n=17$). We assessed remote organ (liver, lung, and brain) effects at 48 h after ischaemia–reperfusion injury. Leukocyte infiltration and apoptosis were assessed using immunofluorescence; cytokines were measured using a cytokine multiplex array. A clinical chemistry analyser measured liver enzymes and gene expression for inflammatory markers was determined by qPCR.
Results
There were no indications of remote organ histopathology or oedema at 48 h after AKI. The number of $TUNEL^+$ apoptotic cells varied between organs but was not affected by AKI, and gene expression of caspase-3 was unaffected. There was no evidence of increased leukocyte infiltration at 48 h in the AKI group. Cytokine concentrations in liver and lung lysate, and gene expression of TNF-α or TGFβ1 were not different between groups. However, animals with AKI had a significantly greater increment in enzymes associated with liver injury (AST, ALT, and ALP) relative to controls that lasted for 24 h but returned to baseline by 48 h.
Conclusions
In our large animal model of AKI, in contrast to small animal models, there was little evidence of any remote organ effects. The greater increment in liver enzymes in animals with AKI is an interesting example of hepato-renal cross-talk but is unlikely to represent acute liver injury.

DOI: 10.1530/endoabs.34.P157

Growth and development

P158

A novel gene affecting the timing of puberty

Sasha Howard[1], Leo Guasti[1], Helen Storr[1], Lou Metherell[1], Anna Cariboni[3], Michael Barnes[1], Claudia Cabrera[1], Karoliina Wehkalampi[2] & Leo Dunkel[1]
[1]Queen Mary, University of London, London, UK; [2]Children's Hospital, Helsinki University Central Hospital, University of Helsinki, Helsinki, Finland; [3]University College London, London, UK.

Background
Disturbances of pubertal timing affect >4% of the population and are associated with adverse health outcomes. Studies estimate 60–80% of variation in pubertal onset is genetically determined, but few genetic factors are known. We hypothesise that causal variants will be low-frequency, intermediate-impact variants and will be enriched in populations at the extremes of normal pubertal timing. Families with constitutional delay in growth and puberty (CDGP) have pubertal onset delayed by >2 S.D., often with an apparent autosomal dominant inheritance pattern.
Methods
Seven highly informative families from our large, accurately phenotyped CDGP cohort were selected for whole exome sequencing. Annotation and filtering of variants produced an extensive list of potential causative mutations that segregate with trait. Variants were ranked on the basis of presence in greater than one family, minor allele frequency <5% or novel, predicted effect on the protein and conservation. Pathway analysis was performed to identify genes with action within the hypothalamic–pituitary–gonadal axis or in linkage disequilibrium ($D' > 0.8$) with loci identified by genome-wide association studies (GWAS) of age-at-menarche.
Results
The 15 top-ranking genes identified all contained non-synonymous missense variants segregating an autosomal dominant pattern. Validation of these 15 genes through targeted re-sequencing in a further 288 CDGP individuals has identified a novel candidate gene, with variants in nine families. This gene has a predicted role in neural outgrowth and is expressed in nasal placode mesenchyme during embryogenesis, suggesting a possible function in GnRH neuronal development. Minimal overlap with GWAS loci was identified. Functional studies of these novel variants are in progress.
Discussion
We describe our strategy for identification of novel causal gene variants from next-generation sequencing data in this common condition. In addition to the exciting finding of a novel gene implicated in the timing of puberty, our results highlight the significant genetic heterogeneity seen in CDGP.

DOI: 10.1530/endoabs.34.P158

P159

Identification and characterisation of human foetal adrenocortical progenitor cells

Jessica Sampson, David Wilson & Ita O'Kelly
University of Southampton, Southampton, UK.

The human foetal adrenal gland (HFA) comprises of two distinct zones; the foetal zone (FZ) and the definitive zone (DZ). The subcapsular DZ has been proposed to contain a population of adrenocortical progenitors that migrate centripetally to populate the FZ. Rodent studies have identified sonic hedgehog (SHH) signalling in a subcapsular non-steroidogenic progenitor population and its disruption during development causes adrenal hypoplasia. The involvement of SHH in the human foetal adrenal gland has yet to be defined. Two background leak potassium channels (K2P3.1 and K2P9.1) are expressed in the rodent adrenal gland and known to be important in aldosterone synthesis. Although their role in the HFA is unknown, K2P3.1 knockout mice exhibit disorganisation of cortical zones thus implicating K2P3.1 in adrenal development. We therefore hypothesised that a HFA progenitor cell population may be identified using markers for the DZ and the SHH pathway. Furthermore, that K2P3.1 and K2P9.1 are expressed within the HFA and play a role in gland development.
RT-PCR identified the expression of SHH pathway components (SHH, PTCH1 SMO, GLI1, GLI2, and GLI3), KCNK3 and KCNK9 within the HFA at the transcript level. K2P3.1 and K2P9.1 were found to be expressed throughout the FZ; however the adrenal capsule and small areas of the DZ appeared to be devoid of K2P3.1. Immunohistochemistry demonstrated SHH and GLI1 (SHH pathway transcriptional activator used as a marker of SHH receiving cells) expression at the periphery of the gland. Furthermore, FACS analysis identified a population of CD56 (DZ marker)- and GLI1-positive cells indicating that cells of the DZ receive a SHH signal. Additionally, inhibition of the SHH pathway with cyclopamine (10 μM), resulted in decreased Ki67-positive DZ cells. Together these data suggests that the SHH pathway is active and maybe involved in maintaining the DZ progenitor population.

DOI: 10.1530/endoabs.34.P159

P160

Relationships between final height and health outcomes in adults with congenital adrenal hyperplasia: United Kingdom Congenital adrenal Hyperplasia Adult Study Executive (CaHASE)

Thang Han[1], Roland Stimson[2], Aled Rees[3], Nils Krone[4], Debbie Willis[5], Gerard Conway[1], Wiebke Arlt[4], Brian Walker[2] & Richard Ross[6]
[1]Department of Endocrinology, University College London, London, UK; [2]Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK; [3]Institute for Molecular and Experimental Medicine, Cardiff, UK; [4]Centre for Endocrinology, Diabetes and Metabolism, Birmingham, UK; [5]Society for Endocrinology, Bristol, UK; [6]Academic Unit of Diabetes, Endocrinology and Metabolism, Sheffield, UK.

Background
Treatment of congenital adrenal hyperplasia (CAH) in childhood focuses on growth and development; however the relationship of childhood treatment with adult health outcomes is not established. We explored this by examining relationships between final height (FH) and cardiometabolic risk in CAH adults.
Methods
Cross-sectional analysis of 65 men (80% salt wasting (SW) and 20% non-SW) and 134 women (74% SW and 26% non-SW), aged 18–69 years. FH was expressed as z-scores adjusted for mid-parental target height (FH_{TH}) or UK population height (FH_{UK}).
Results
FH correlated inversely with age (men $\beta = -0.30$, $r = -0.38$, $P < 0.01$ and women $\beta = -0.18$, $r = -0.26$, $P < 0.01$); older men and women were 3 and 2 cm, respectively, shorter per decade. FH z-scores were -2 (men) and -1 (women) below TH and both sexes had FH z-scores -1 below the reference population ($P < 0.01$). In women, FH was shorter in non-SW than SW classic CAH ($P < 0.05$) and in moderately affected genotype group B women than either more severely affected groups Null and A ($P < 0.01$) or mildest group C ($P < 0.001$). Classic CAH men and women diagnosed after 1-year-old were also shorter ($P < 0.05$). In women, the shortest group (lowest FH_{TH} tertile) was 3.6 times (95% CI 1.2–10.8, $P < 0.05$) more likely to have hypertension than the tallest group (highest FH_{TH} tertile). Similar observations were made using FH_{UK} tertiles. FH did not associate with insulin sensitivity, lipid profile, or waist circumference.
Conclusions
Adult CAH patients remain shorter than predicted although height prognosis has improved over time suggesting better management in childhood. Patients diagnosed late with moderate severity CAH (e.g. after 1-year-old with pseudo-precocious puberty) are shorter as adults, and in women short stature was associated with adult hypertension. We suggest this group are likely to be exposed to high androgens and/or to excessive glucocorticoid treatment in childhood, with consequent reduced FH and potential programming of hypertension.

DOI: 10.1530/endoabs.34.P160

P161

CUL7, OBSL1 and CCDC8 modulate alternative splicing of exon 11 of the insulin receptor gene

Daniel Hanson, Graeme Black & Peter Clayton
University of Manchester, Manchester, UK.

Background
The insulin receptor (INSR) is alternatively spliced in a developmental and tissue specific manner into two isoforms, IR-A and IR-B. IR-A excludes exon 11 and is widely expressed whereas IR-B includes exon 11 and is expressed in insulin sensitive tissues. The severe short stature disorder 3-M syndrome is caused by mutations in *CUL7*, *OBSL1* and *CCDC8* and we have recently associated these proteins with the major mRNA splicing pathways including the heterogeneous nuclear ribonucleoprotein (HNRNP) complex.
Objective
To determine if the 3-M proteins regulate the alternative splicing of *INSR*.
Methods
We co-transfected expression vectors for the 3-M genes (*CUL7*, *OBSL1* and *CCDC8*) and an INSR minigene into HEK293 cells, the INSR minigene was also transfected into fibroblast cells from healthy controls and 3-M syndrome patients. Analysis of exon 11 splicing was determined by RT-PCR and calculating alteration of the ratio IR-B/IR-A ratio by gel densitometry.

Results
Over-expression of the 3-M genes in HEK293 cells causes a decrease in exon 11 inclusion resulting in a reduction in the ratio of IR-B/IR-A expression ($P<0.001$). While reduced expression of the 3-M genes in patient cells has the opposite effect with an increase in exon 11 inclusion and an increase in the ratio of IR-B/IR-A expression ($P<0.001$).
Conclusion
Alternative *INSR* splicing has been previously associated with different HNRNP proteins and we now demonstrate that the interaction of the 3-M proteins within this complex can also modulate exon 11 splicing. This may in part explain the reduced growth seen in short stature patients as IR-A exhibits the mitogenic effects of insulin while IR-B exhibits its metabolic effects. Conversely elevated expression of *CUL7* and IR-A have been associated with increased risk of tumours and cancer progression suggesting a more widespread role of the 3-M genes as growth regulators beyond stature.

DOI: 10.1530/endoabs.34.P161

P162

Abstract Withdrawn.

DOI: 10.1530/endoabs.34.P162

P163

C-type natriuretic peptide regulates growth and pituitary development in the zebrafish (*Danio rerio*)

Andrew Lessey[1], Samantha Mirczuk[1], Imelda McGonnell[2] & Robert Fowkes[1]
[1]Endocrine Signalling Group, Department of Comparative Biomedical Sciences, The Royal Veterinary College, London, UK; [2]Department of Comparative Biomedical Sciences, The Royal Veterinary College, London, UK.

C-type natriuretic peptide (CNP), the predominant natriuretic peptide in the pituitary, is associated with neuroendocrine development and function. Our previous studies show that genes encoding CNP (*nppc2*, *cnp3*, *nppc4*, and *nppcl*) are expressed during pituitary development in humans and zebrafish (*Danio rerio*), however a precise role for CNP in the pituitary has yet to be elucidated. Using the versatile zebrafish, we explored *in vivo* effects of exposing developing embryos to exogenous CNP. WT Tupfel long fin (TL) zebrafish embryos were collected from staged matings and exposed to 100 nM CNP-22 for 24, 48 or 72 hours post-fertilisation (hpf). Biometric analyses revealed a significant reduction in body length in embryos exposed to CNP across the developmental time course, along with a delayed onset of melanosome aggregation compared to untreated controls. Total RNA was subsequently extracted from treated and control embryos and simultaneous gene expression of the natriuretic peptide system in zebrafish (*nppa* (ANP), *nppb* (BNP), *nppc2*, *cnp3*, *nppc4*, *nppcl* (CNPs), *npr1a*, *nprA* (GC-A), *nprB* (GC-B), *nprC* (NPR3) *corina* (corin), *furina* and *furinb* (furins)) and phenotypic indicators of pituitary cell lineage (*cga* (α-subunit), *gh1* (GH), *pomca* (proopiomelanocortin), *pou1f1* (pit-1), *smtlβ* (somatolactin) and *tshβ* (thyroid stimulated hormone)) were examined using a multiplex qRT-PCR assay and GenomeLab GeXP analysis system. *nppc4* was significantly decreased at 24 hpf ($P<0.05$) but increased at 48 hpf ($P<0.01$), whereas, *nppcl* was decreased at 48 and 72 hpf ($P<0.01$ and 0.05 respectively). Both *npra* and *pomca* were reduced at 48 hpf ($P<0.05$ and 0.01 respectively). To investigate the effects of elevated CNP exposure on cGMP accumulation, dispersed primary zebrafish embryo cultures were exposed to 100 nM CNP or ANP for up to 60 min. Both CNP and ANP increase cGMP accumulation over 60 min. Collectively, these data suggest that CNP has a role in the regulation of growth and pituitary gene expression during zebrafish development.

DOI: 10.1530/endoabs.34.P163

P164

Distinct gene expression is associated with epigenetic and growth-related network modules in relation to gender differences in the timing of the pubertal growth spurt

Chiara De Leonibus[1], Pierre Chatelain[2], Peter Clayton[1] & Adam Stevens[1]
[1]University of Manchester, Manchester, UK; [2]Université Claude Bernard, Lyon, France.

Background
The return to active long bone growth in puberty is a distinctly human event[1] and occurs ~2 years earlier in girls compared to boys. Evolutionarily conserved networks of genes are associated with the developmental phases of childhood in multiple tissues[2], implying the existence of a genetic program that controls the pubertal return to growth.
Objectives
To identify biological functions associated with gender and age-related gene expression in puberty.
Methods
We conducted gene expression (GE) analysis on a library of datasets from normal children with age annotation, collated from the NCBI Gene Expression Omnibus (GEO) and EBI Array express databases. A primary data set was generated using cells of lymphoid origin ($n=87$, 43 boys and 44 girls). For analysis in other tissue data sets were available from muscle ($n=14$), brain ($n=30$) and conjunctival epithelium ($n=18$). GE associated with puberty was assessed using ANOVA ($P<0.01$) with hierarchical clustering. Age-related GE associated with gender was defined using rank regression ($P<0.01$). Human interactome network modules associated with GE were identified using the WEB-based GEne SeT AnaLysis Toolkit (WebGestalt)[3].
Results
A cluster of 626 gene probes were associated with puberty independent of gender ($P<0.01$). Age-related differential GE was identified related to gender (813 gene probes). An overlap of 235 gene probes were both associated with gender and puberty (28.9%). Interactome network modules were mapped to this overlap and were associated with the growth hormone signalling pathway ($P=2.0\times10^{-4}$) and histone demethylase activity ($P=8.1\times10^{-2}$). These observations were confirmed in pubertal associated GE in muscle, brain and conjunctival epithelium.
Conclusions
Specific GE profiles were identified in relation to gender differences in puberty. Network analysis showed that biological pathways involved in puberty included growth and epigenetic related clusters of genes. The genes identified in this study could potentially act as markers of the timing and tempo of puberty.
References
1. Bogin B. Evolutionary perspective on human growth. *Annu Rev Anthropol* 1999 **28** 109–153.
2. Stevens A, Hanson D, Whatmore A, Destenaves B, Chatelain P & Clayton P. Human growth is associated with distinct patterns of 3 gene expression in evolutionarily conserved networks. *BMC Genomics* 2013 **14** (1) 547.
3. Wang J, Duncan D, Shi Z & Zhang B. WEB-based GEne SeT AnaLysis Toolkit (WebGestalt): update 2013. *Nucleic Acids Res* 2013 **41** W77–W83.

DOI: 10.1530/endoabs.34.P164

P165

Altered frequency of sequence variants in growth-related genes in children with short stature

Philip Murray[1,2], Adam Stevens[1], Daniel Hanson[1], Andrew Whatmore[1], Mireille Bonnemarie[3], Laurent Naudin[3], Pascale Dutailly[3], Patrice Denefle[3] & Peter Clayton[1,2]
[1]University of Manchester, Manchester, UK; [2]Royal Manchester Children's Hospital, Manchester, UK; [3]IPSEN Pharma SAS, Boulogne-Billancourt, France.

Background
Many children with short stature (defined as height SDS <-2 S.D.) have no identified cause for their growth impairment and are classified as either small for gestational age (SGA) or idiopathic short stature (ISS) depending on birth size. Adult height is a polygenic trait and has been associated with >180 single nucleotide polymorphisms (SNPs) to date. We hypothesized that sequence variants (SNPs or insertion/deletions (indels)) in candidate genes (associated with short stature disorders, growth pathways or adult height) may contribute to the growth impairment in ISS and SGA children.

Study population
263 children and 263 ethnically matched controls from nine European countries classified as either ISS or SGA and enrolled in the EPIGROW study in whom next generation sequencing of 232 candidate genes had been performed.
Methods
Analysis of the sequencing data to determine genes with a different frequency of SNPs or indels between the patient and control cohorts. SNP/indel frequency was considered to be different where a Benjamini–Hochberg adjusted P value from a χ^2 test was <0.05. SNP/indel frequency was assessed for both carriage of SNP/indel (homozygous + heterozygous vs normal) and carriage of homozygous SNP/indel (homozygous vs heterozygous + normal).
Results
30 genes were identified where SNP carriage frequency (homozygous + heterozygous) was significantly different. In patients SNP frequency was increased for 12 genes and decreased for 18 genes. These included *IGFALS* (↓), *HRAS* (↑), *STAT5b* (↓) and *FANCA* (↓) which are associated with short stature conditions, *MAP2K1* (↑) and *SOCS1* (↑) associated with growth pathways and *SDR16A5* (↑) associated with adult height. No genes were identified in which the frequency of carriage (homozygous + heterozygous) of an indel significantly differed. For one gene *RPS6KA6* (a protein kinase involved in growth factor signalling), carriage of homozygous indels were more common in patients ($P=0.001$).
Conclusions
There are growth-related genes in which sequence variant frequency were significantly different between children with short stature and controls. Combinations of functional variants in these genes may contribute to growth impairment.

DOI: 10.1530/endoabs.34.P165

P166

Differential effects of grazing and meal feeding on skeletal growth and femoral strength in male rats

Thomas W Tilston[1], Anna L Hopkins[1], Richard C Brown[1], Bradley Arms-Williams[1], Sam L Evans[2] & Timothy Wells[1]
[1]School of Biosciences, Cardiff University, Cardiff, UK; [2]School of Engineering, Cardiff University, Cardiff, UK.

The pattern of food consumption has a profound influence on metabolic hormone secretion, but until recently only crude manipulations of feeding events have been possible in rodents. Using a CLAMS-based system to overcome this problem, we have investigated the effect of 3 weeks of grazing (consumption of 1/24th of the total daily food intake of *ad libitum*-fed controls every 30 min during the dark phase (18.00–06.00 h)) and meal feeding (three 1-h periods of *ad libitum* food access at 18.00, 23.30 and 05.00 h) on skeletal growth and femoral strength in 6-week-old male Sprague–Dawley rats.
Grazing and meal-fed rats showed a 15% reduction in cumulative food intake, but only meal-fed animals exhibited less weight gain than *ad libitum*-fed controls. Similarly, although body length was reduced by 3% in both grazing and meal-fed rats ($P<0.05$), tibial and femoral length was only reduced in meal-fed animals (3% lower; $P<0.05$). Overall cortical strength (as assessed by three-point bending) was unaffected with either feeding pattern, but grazing rats showed a 24% increase in the geometric component of strength (2nd moment of area; $P<0.05$), due largely to a 35% increase in the thickness of the posterior cortex ($P<0.05$ vs meal-fed).
Profiles of circulating total ghrelin (as assessed by automated blood sampling) were identical in grazing and meal-fed rats, both feeding patterns eliciting the expected anticipatory rise in ghrelin prior to the commencement of the dark (feeding) phase. Overall GH secretion (area under curve), baseline levels, pulse height and pulse frequency were unaffected in grazing rats, but the daily nadir in peak heights was shifted into the early dark phase.
Given the short period of exposure to patterned feeding, the subtle effects observed in the growth and biomechanical performance of bone are remarkable, suggesting that the pattern of daily food consumption in adolescence may influence the three-dimensional shape of bone during the growth phase, potentially resulting in longer-term consequences for adult bone health.

DOI: 10.1530/endoabs.34.P166

Neoplasia, cancer and late effects

P167

Calycosin suppresses breast cancer cell growth via ERβ-mediated inhibition of IGF1R pathway

Jian Chen & Jing Tian
Guilin Medical University, Guilin, China.

We previously reported that calycosin, a natural phytoestrogen structurally similar to estrogen, successfully triggered apoptosis of ER-positive breast cancer cell line, MCF-7. To better understand the antitumor activities of calycosin against breast cancer, besides MCF-7 cells, another ER-positive cell line T-47D was analyzed here, with ER-negative cell lines (MDA-231 and MDA-435) as control. Notably, calycosin led to inhibited cell viability and apoptosis only in ER-positive cells, particularly in MCF-7 cells, but no such effect was observed in ER-negative cells. Thus MCF-7 cells were chosen to further identify the possible link between calycosin and ER signaling. After the treatment of calycosin, the expression levels of ER β (ERβ) were greatly increased in MCF-7 cells, whereas the expression of ER α (ERα) remained relatively constant. Moreover, accompanied by upregulation of ERβ, successive changes in downstream signaling pathways were found, including inactivation of IGF-1R, then stimulation of p38 MAPK and suppression of the serine/threonine kinase (Akt), and finally poly(ADP-ribose) polymerase 1 (PARP-1) cleavage. However, the other two members of the MAPK family, extracellular signal-regulated kinase (ERK) 1/2 and c-Jun N-terminal kinase (JNK), were not consequently regulated by downregulated IGF-1R, indicating ERK 1/2 and JNK pathways were not necessary to allow proliferation inhibition by calycosin. Taken together, our results indicate that calycosin tends to inhibit growth and induce apoptosis in ER-positive breast cancer cells, which is mediated by ERβ-induced inhibition of IGF-1R, along with the selective regulation of MAPK and phosphatidylinositol 3-kinase (PI3K)/Akt pathways.

DOI: 10.1530/endoabs.34.P167

P168

A challenging case of hypokalaemic Cushing's crisis

Mary Dunne[1], Andrew Solomon[1,2] & Francesca Swords[3]
[1]Bedford Hospital NHS Trust, Bedford, UK; [2]East and North Herts NHS Trust, Stevenage, UK; [3]Norfolk and Norwich NHS Foundation Trust, Norwich, UK.

Background
Cushing's syndrome derived from paraneoplastic ectopic ACTH production can present in dramatic fashion. We describe such a case that presented with severe hypokalaemia, involving significant diagnostic and management challenges.
The case
A 66-year-old female was referred acutely to hospital by her GP after feeling weak and lethargic. She had profound hypokalaemia of 1.5 mmol/l (3.5–5.3) with normal renal function and had been normokalaemic 6 weeks earlier. Past history included previous localised breast cancer and a metastatic bladder carcinoma – with evidence of findings in the lungs and liver – the latter had undergone partial resection. Her medication was furosemide 20 mg (which had been started a week ago), amlodipine 5 mg, simvastatin 40 mg, and anastrazole 1 mg. On examination: pulse 90 bpm, BP 140/80, and she had massive generalised oedema. She was admitted to critical care. Investigations showed ABG pH 7.61 (7.35–7.45) and HCO_3 56 (22–30). Random glucose was 6.8, prolactin 230, TSH 0.72 and FT_4 12.0. Emergency management was initially intravenous potassium replacement. An endocrine consultation noted a clearly Cushingoid appearance. Further investigations revealed 0900 cortisol of 5957 nmol/l and after an overnight 1 mg dexamethasone suppression test 5595 nmol/l. An aldosterone:renin ratio was normal at 320. The primary bladder tumour histology was reported as showing small cells exhibiting neuroendocrine differentiation with strongly CD56++ and Synaptophysin++. In order to suppress her cortisol, she was commenced on metyrapone titrated to 100 mg tds with clinical and biochemical benefit. She was later transferred to a hospice.
Conclusion
This patient presented with a hypokalaemic Cushing's crisis and profound metabolic alkalosis exacerbated by recent exposure to furosemide. Ectopic ACTH causes <10% of cases of Cushing's and only very few of those have been reportedly due to small cell carcinoma arising from the bladder. This case thus provided profound insights into the care of such patients.

DOI: 10.1530/endoabs.34.P168

P169

Investigating neuroendocrine markers of small cell lung cancer

Thomas Layton
University of Manchester, Manchester, UK.

Small cell lung cancer (SCLC) is a common and devastating disease. SCLC tumours contain a neuroendocrine cell population that exhibit ectopic hormone production in a minority of patients. The aim of this study was to investigate a panel of neuroendocrine peptides as potential biomarkers of SCLC, including pro-opiomelanocortin, neuron specific enolase, chromogranin A and neural-cell adhesion molecule. Immunohistochemistry methods were used to examine neuroendocrine peptides in five human lung cancer cell lines (CORL24, CORL47, DMS79, H526, and A549) and a novel murine subcutaneous SCLC xenograft tumour. Liver, lung and brain samples were taken from xenograft mice to identify possible metastasis. ELISA measured POMC secretion and expression in cell lines during incubation and a western blot quantified chromogranin A levels in all cell lines and xenograft tumours. Results confirmed a significant neuroendocrine cell population within SCLC xenograft tumours and cell lines. Additionally, POMC secretion proved to be a marker of SCLC growth *in vitro* in CORL24 and DMS79. H526, CORL47 and A549 did not secrete POMC. Xenograft tumours appeared invasive with infiltration of surrounding connective tissue, but no metastases were present in liver, lung and brain samples. This study validates further investigation into the neuroendocrine phenotype in SCLC, and POMC as a potential biomarker for the disease.

DOI: 10.1530/endoabs.34.P169

P170

Comparison of HbA1c and oral glucose tolerance testing for the diagnosis of patients with and at risk of diabetes among long-term bone marrow transplant survivors

Sowmya Gururaj, Victoria Cartwright, Samiul Mostafa, Ann Hunter & Helena Gleeson
University Hospitals of Leicester, Leicester, UK.

Background
Bone marrow transplant (BMT) survivors are at increased risk of metabolic syndrome and developing type 2 diabetes (T2DM). Measurement of HbA1c is increasingly used in recent years for the diagnosis of T2DM. An HbA1c level of $\geq$6.5% (48 mmol/mol) is considered as a diagnostic cut-off for T2DM and an HbA1c between 6.0–6.4% (42–47 mmol/mol) is considered as at risk of T2DM. We aimed to look at the diagnostic performance of HbA1c and oral glucose tolerance testing (OGTT, diagnosed using WHO 1999 criteria) in identifying patients with/at risk of T2DM among long-term BMT survivors.
Materials and methods
Our study identified 129 consecutive allogeneic BMT survivors who underwent BMT at Leicester Royal Infirmary between January 1986 and December 2007. 68.2% ($n=88$) of the above patients were offered an appointment at late effects clinic between January 2010 and December 2012. Those who did not attend ($n=30$), those with known DM-2 ($n=9$) and who had incomplete investigations ($n=4$) were excluded from the study group ($n=45$). Data regarding demographics, medical history and drug history were recorded on the day of the visit. Factors[1] which could influence HbA1c such that it no longer reflects true glycaemic levels were investigated including levels of haemoglobin, ferritin, urea, liver function tests, and aspirin use. OGTT and HbA1c results on clinic day were reviewed.
Results
The final analysis consisted of 45 people. HbA1c results revealed 8.8% ($n=4$) in T2DM range (HbA1c 8.31%$\pm$1.36 (mean$\pm$S.D.)) while 24.4% ($n=11$) were at risk of T2DM (HbA1c 6.19%$\pm$0.13). The OGTT results revealed 8.8% ($n=4$) with DM-2, 2.2% each with impaired glucose tolerance (IGT) ($n=1$) and impaired fasting glucose (IFG) ($n=1$) while remaining ($n=39$) with normal glucose tolerance.
All the patients with abnormal OGTT (DM-2, IGT and IFG) also had abnormal HbA1c results. All the patients with HbA1c $\geq$6.5% 03/02/2014had abnormal OGTT results with 50% of OGTT in T2DM range while 25% with IGT and 25% with IFG. Interestingly, patients with abnormal HbA1c in at risk range, the majority (91%, $n=10$) had normal glucose tolerance while the remaining 9% had IFG thus showing a large discordance in this sub-group.
Discussion
There is a large discordance between HbA1c and OGTT results in diagnosing BMT survivors at risk of T2DM**.** Use of an OGTT could result in a lower tendency to diagnose BMT survivors at risk of T2DM compared to an HbA1c test. Interestingly this discordance is more marked and contrary to what some other studies which focus on the general population[2], suggesting our finding may be a specific to BMT survivors. Given the high risk of cardiovascular mortality in this group, underdiagnoses of at risk T2DM group would delay prompt initiation of preventative measures.
References
1. Gallagher EJ, Bloomgarden ZT & Le Roith D. Review of hemoglobin A1c in the management of diabetes. *J Diabetes* 2009 **1** 9–17.
2. Farhan S, Jarai R, Tentzeris I, Kautzky-Willer A, Samaha E, Smetana P, Jakl-Kotauschek G, Wojta J & Huber K. Comparison of HbA1c and oral glucose tolerance test for diagnosis of diabetes in patients with coronary artery disease. *Source*. Third department of Medicine, Cardiology an Emergency Medicine, Wilhelminen Hospital, Montleartstrasse 37, 1160 Vienna, Austria.

DOI: 10.1530/endoabs.34.P170

P171

Neuroendocrine biomarkers change with treatment in small cell lung cancer

Suzanne Meredith, Jennifer Bryant, Muhammad Babur, Philip Riddell, Kaye Williams & Anne White
University of Manchester, Manchester, UK.

Small cell lung cancer (SCLC) is distinguished by its neuroendocrine phenotype and secretion of hormone biomarkers. Tumours initially respond well to therapy but almost invariably relapse with a therapy-resistant phenotype. We have previously described pro-opiomelanocortin (POMC) as a potential biomarker of SCLC. In this study, we investigated how POMC acts as a biomarker of tumour response to treatment.
SCLC cells (DMS 79) were injected subcutaneously into nude mice and tumours were irradiated for 3, 5 or 10 consecutive days at 2 Gy or left untreated. Circulating POMC strongly mimicked tumour growth in untreated animals. After irradiation, tumours decreased in size and subsequently grew back to the same size as untreated tumours. POMC concentrations mimicked tumour size. However, in those tumours receiving the high dose irradiation, circulating POMC, tumour POMC protein and gene expression were all significantly decreased. Other neuroendocrine markers including neuron specific enolase (NSE) and neural cell adhesion molecule (N-CAM) were unchanged.
To determine whether this change in biomarker expression was associated with irradiation-resistance occurring, DMS 79 cells were made resistant by repeated exposure *in vitro*. POMC expression was again significantly lower in the irradiation-resistant cells with NSE, N-CAM and chromogranin A expression unchanged. In addition, irradiation-resistant cells showed a drastically altered morphology, increased proliferation and an upregulation of genes associated with a more mesenchymal phenotype.
The changes to the neuroendocrine phenotype in response to irradiation are complex, as the decrease in POMC *in vivo* and *in vitro* occurred in the absence of changes in other neuroendocrine markers. This indicates that extensive characterisation of biomarkers is necessary to understand their plasticity and the implications for their use. The transition to a more mesenchymal phenotype after irradiation suggests that despite the obvious advantage of treating SCLC with radiotherapy, the remaining cells may have a greater propensity to metastasise.
The changes to the neuroendocrine phenotype in response to irradiation are complex, as the decrease in POMC *in vivo* and *in vitro* occurred in the absence of changes in other neuroendocrine markers. This indicates that extensive characterisation of biomarkers is necessary to understand their plasticity and the implications for their use. The transition to a more mesenchymal phenotype after irradiation suggests that despite the obvious advantage of treating SCLC with radiotherapy, the remaining cells may have a greater propensity to metastasise.

DOI: 10.1530/endoabs.34.P171

P172

Late presentation predicts endocrine dysfunction in patients with Langerhans cell histiocytosis: a retrospective analysis of the West of Scotland LCH service 1998–2012

Aishah Coyte[1], Dermot Murphy[2], Milind Dattatraya Ronghe[2], Jairam Sastry[2], Faisal Ahmed[1,3] & Mohamad Guftar Shaikh[1,3]
[1]University of Glasgow, Glasgow, UK; [2]Department of Oncology, Royal Hospital for Sick Children, Yorkhill, Glasgow, UK; [3]Department of Endocrinology, Royal Hospital for Sick Children, Yorkhill, Glasgow, UK.

Introduction
Langerhans cell histiocytosis (LCH) is a rare condition primarily affecting children. It is characterised by clonal proliferation of Langerhans cells. Disease severity is dependent on the type and number of organs involved in addition to focality. Disease aetiology remains unclear. We aimed to record the incidence and characteristics of West of Scotland LCH patients with a focus on predictive factors for endocrine dysfunction.
Method
Consecutive diagnoses of LCH, at The Royal Hospital for Sick Children Glasgow, between January 1998 and December 2012 were identified. Patient notes were used to collect information on age, sex, postcode (Scottish index of deprivation-SIMD: one-most deprived, six-least deprived), ethnicity, systems involved, signs and symptoms of presentation, disease progression, treatment and current status.
Results
23 patients were identified. Median age of diagnosis was 2.7 years (range: 1 month–15 years). 52% ($n=12$) were female. Over half of patients 57% ($n=13$) have SIMD scores of 1/2. 82% of patients were Caucasian, 13% South Asian and 4% mixed origin. The head was the most common site of presentation. There was 1 mortality in the cohort. Common symptoms at presentation included skin rashes, lumps and musculoskeletal pain. All patients with high risk organ involvement (spleen, liver, hematopoietic system or lung) were girls. Endocrine dysfunction, with diabetes insipidus being most common, was seen in 35% ($n=8$) . 62.5% ($n=5$) were diagnosed with diabetes insipidus alone. Patients with endocrine dysfunctions had a longer symptom interval compared to those without endocrine dysfunction; with 62.5% ($n=5$) diagnosed after 4 months compared to 13.3% ($n=2$) without endocrine dysfunction ($P=0.052$).
Conclusion
Females are more likely to develop high risk organ disease. Deprivation is associated with an increased incidence of LCH, which has not been previously reported. A third of paediatric LCH patients develop endocrine dysfunction and these patients have a longer symptom interval.

DOI: 10.1530/endoabs.34.P172

P173

The first reported mutations in the pituitary tumor-transforming gene binding factor

Waraporn Imruetaicharoenchoke, Martin Read, Joel Smith, Vicki Smith, Bhavika Modasia, Vikki Poole, Rachel Watkins, Franklyn Jayne, Kristien Boelaert & Christopher McCabe
University of Birmingham, Birmingham, UK.

PBF is a ubiquitous glycoprotein which is over-expressed particularly in endocrine and endocrine-related cancers. Previously classified as a proto-oncogene, 11 substitution-missense mutations of PBF have now been reported in tumours from patients with ovarian, prostate and colorectal cancers via the COSMIC database, suggesting PBF may in fact be an oncogene. We have therefore examined the biological implications of all 11 mutations. Substitution mutations, which occurred across the 180 amino acid structure of PBF, with a potential hot-spot at residue 106, were re-created in tagged (HA and FLAG) and untagged constructs. Modelling of all mutants using I-TASSER identified potentially severe alterations in 3D structure for mutants W59F, R87C, S103L, G106R, G106W and R140W. SIFT predictions identified that all mutations except G106V would result in significant changes in protein function. Western blotting revealed that mutations C51R and G106R inhibited PBF dimerisation and glycosylation *in vitro*. Anisomycin half life studies in SW1736 thyroid and MCF7 breast cancer cells revealed that R87C and S103L mutations resulted in unchanged protein stability compared to WT (half life ~24 h), whereas C51R, W59F, G106R, G106V, G106W and R146W mutants were less stable, ranging from approximately 6 to 9 h. Interestingly, different tumour mutations at residue 106 revealed very different biochemical properties of the protein, with G106R showing significantly reduced glycosylation, dimerisation and half life, whereas G106V and G106W demonstrated WT levels of dimerization, and with G106V having 50% greater stability than G106W. This is the first description of mutations in PBF. Based on our initial biochemical characterisation of 3D protein structure, glycosylation, dimerisation and stability, we are now determining the functional consequences of these initial mutations on the known actions of PBF in endocrine neoplasia, to address the question of whether PBF mutations are oncogenic *in vivo*.

DOI: 10.1530/endoabs.34.P173

P174

Bromodomain inhibitors reduce proliferation and increase apoptosis of human neuroendocrine tumour cells

Kate E Lines[1], Mark Stevenson[1], Katherine U Gaynor[1], Paul J Newey[1], Sian E Piret[1], Hannah Lingard[2], Panagis Filippakopoulos[2], Susanne Muller[2], Simona Grozinsky-Glasberg[3], Ashley Grossman[1], Stefan Knapp[2], Chris Schofield[2], Chas Bountra[2] & Rajesh V Thakker[1]
[1]Academic Endocrine Unit, OCDEM, University of Oxford, Oxford, UK; [2]Structural Genomics Consortium, University of Oxford, Oxford, UK; [3]Neuroendocrine Tumour Unit, Endocrinology & Metabolism Service, Department of Medicine,Hadassah-Hebrew University Medical Center, Jerusalem, Israel.

Pancreatic neuroendocrine tumours (NETs) are reported to show frequent mutations in chromatin remodelling genes, while pituitary NETS have alterations in histone modification. Histone modifications, and specifically acetylated residues on histone tails are recognised by members of the bromo and extra terminal (BET) protein family, via their bromodomains, causing alterations in the transcription of growth stimulating genes. BET bromodomain inhibitors have been demonstrated to successfully reduce tumour growth in mouse models, including those for nuclear protein in testis (NUT)-midline carcinoma, non-small cell lung cancer, leukaemia and glioblastoma. We therefore assessed the efficacy of two pan-BET inhibitors, JQ1 and PFI-1, on proliferation, apoptosis and senescence of three human NET cell lines (BON-1 cells derived from a pancreatic NET, and H727 and H720 cells derived from lung NETs) and one mouse cell line (AtT20 cells derived from the pituitary). Proliferation was assessed by the Cell Titre Blue fluorescent assay, apoptosis by caspase 3/7 cleavage, and cellular senescence by β-galactosidase (X-gal) staining. Our results show that JQ1 and PFI-1 had significant effects on proliferation, apoptosis and senescence. Thus, in all four cell lines JQ1 reduced proliferation by up to 95% ($P<0.0001$), with an average IC_{60} of 36 nM, while PFI-1 reduced proliferation by up to 40% ($P\leq0.0002$), with an average IC_{60} of 800 nM. JQ1 also significantly increased apoptosis by up to 3.5-fold ($P<0.0005$) in all four cell lines, and PFI-1 increased apoptosis in BON-1 and H720 cells by up to twofold ($P<0.05$). JQ1 significantly increased cellular senescence, of BON-1 and H727 cells, by up to sixfold ($P<0.0001$) and PFI-1 significantly increased senescence of BON-1 cells by twofold ($P>0.0005$). Thus, our data demonstrate that BET protein inhibitors may represent potential compounds for the treatment of neuroendocrine tumours.

DOI: 10.1530/endoabs.34.P174

P175

Adrenocortical cancer: rare but gloomy cause of adrenal lesions

Agnieszka Falinska, Tanaz Vakilgilani, David Woods, Yong Yong Ling, Raj Tanday & Jeannie F Todd
Imperial Centre for Endocrinology, Hammersmith Hospital, London, UK.

We present a case of 50-year-old female who presented to a local hospital with sudden onset severe sharp left flank pain. Urgent CT revealed a mass in the left adrenal gland which was thought to be a supra-renal bleed. It measured 6 cm at this point. She was discharged with paracetamol and re-assured. As she was not feeling well, she had an US kidney in private sector 1 month later revealing static appearance of presumed haematoma. Following the period of observation to allow haematoma to re-absorb, she had MRI repeated 6 months after initial presentation. It confirmed 11 cm growing mass in left adrenal. At this point, the patient was referred to endocrine services. On further questioning she admitted to recent onset of hirsuitism and her androgens levels were raised (testosterone 20.7 nmol/l, normal <2.5 nmol/l). Additionally she failed overnight dexamethasone suppression tests (0900 h cortisol 92 nmol/l). Her blood pressure was elevated.
Pre-surgery CT adrenal revealed on-going growth of the mass reaching 12.8 cm. She had total left adrenalectomy and nephrectomy and macroscopically she was clear of cancer. Histology revealed adrenal cortical cancer with invasion of vessels. Post operatively her adrenal androgens were suppressed consistent with successful clearance of the tumour. She was referred to oncologists and has been

started on mitotate which she tolerating it reasonably well. Post-operative CT scan shows no evidence of recurrence.
Adrenocortical carcinoma has an estimated incidence of around 0.5–2 new cases per million people per year. It is more common in female in the fourth to fifth decades of life. It accounts for around 2–5% of cases of patients with adrenal incidentaloma. This case highlights the importance of appropriate referral of suspected adrenal lesion to endocrinology department to assess functionality and surveillance. Although rare, adrenocortical cancer should always be considered in the differential diagnosis of adrenal lesion over 4 cm.

DOI: 10.1530/endoabs.34.P175

P176

Metastatic paraganglioma with unknown genetics: to screen or not to screen the family?

Agnieszka Falinska, Tanaz Vakilgilani, David Woods, Raj Tanday, Yong Yong Ling & Jeannie F Todd
Imperial Centre for Endocrinology, Hammersmith Hospital, London, UK.

We present a family of a 45-year-old patient who presented to hospital acutely unwell with metastatic paragangliomas. Unfortunately due to rapidity of his presentation, no genetic testing was performed. He was found to have 5 cm right carotid body tumour. His urine collections confirmed raised (seven times normal) 24 h urine metanephrines 24.55 μmol (normal <3.47 μmol). Shortly after the initial diagnosis he was found to have extensive vertebral body metastases in cervical and thoracic spine, liver metastases and widespread lymphadenopathy. Lymph node biopsy confirmed metastatic paraganglioma. He originally came from India and there is no other family history of pheochromocytoma or paraganglioma other that in this individual. Both his parents died 20 years ago of unknown cause and he has three brothers and three sisters who live in London, India, and USA. Two of his siblings are patients in our clinic. The younger brother is 43 years and clinically he is well. He has dominant thyroid nodule on the background of multinodular goitre with repeated FNAs confirming benign lesion. His older sister is 63 years old and suffers from chronic myeloid leukaemia.
Paragangliomas are rare and up to 30% are genetically determined. Multiple and metastatic paragangliomas are more common (17–85%) in hereditary syndromes compared to only 1.2% of sporadic cases. Malignancy rates are highest for paragangliomas that arise in the setting of an inherited mutation in the B subunit of the succinate dehydrogenase (SDHB) gene.
Genetic testing is guided by the family history and clinical findings and is usually offered to all subjects undergoing surgery for head and neck paraganglioma and /or to the family of an individual with confirmed genetic mutation.
Our cases highlight important management issues. Should his siblings be tested for a susceptibility gene? If yes, genetic testing for which mutations? If genetic screen is negative, can the individuals be safely discharged or should they undergo long term biochemical screening? If not tested, should they be followed up as having presumed susceptibility gene or discharged form regular follow-up?

DOI: 10.1530/endoabs.34.P176

P177

Clinical dilemmas in diagnosing pheochromocytoma

Agnieszka Falinska, Tanaz Vakilgilani, Raj Tanday, Yong Yong Ling & Jeannie F Todd
Imperial Centre for Endocrinology, Hammersmith Hospital, London, UK.

A 43-year-old gentleman was referred from a local hospital with biochemical and radiological suspicion of pheochromocytoma. He admitted to a 10-year history of palpitations, flushing, sweating, and tremors. He was intermittently feeling stressed, anxious and angry with mood swings. He had difficulty sleeping and suffered from headaches. He was treated for hypertension and 10 years prior he was admitted to the local hospital with malignant hypertension. He was drinking up to 40 units alcohol/week. There was a strong family history of ischaemic heart disease and hypertension. He was started on doxazosin as he did not tolerate phenoxybenzamine.
Results from referring hospital shown mildly raised 24 h urinary metanephrines. His initial CT imaging was normal but an MIBG scan suggested increased diffuse uptake in the left adrenal gland reported as consistent with phaeochromocytoma. Repeat 24 h urine metanephrines were again mildly raised on two out of three occasions. His urine catecholamines were within normal range.
Repeat MIBG again confirmed diffuse uptake in the left adrenal area and CT component showed normal adrenal glands. Images were discussed at Endocrine MDT and it was felt that this asymmetrical uptake could be physiological. He underwent clonidine suppression test which showed plasma noradrenaline 2.3 nmol/l at beginning of the test suppressing to 1.4 nmol/l (NR $<$ 5 nmol/l) bringing the diagnosis of phaeochromocytoma into question. He was referred for sleep study and to psychologist. Although it was felt that a phaeochromocytoma was unlikely, an expectant approach was adopted with plan for repeat interval scan at 6 months.
Repeat adrenal MRI confirmed a 9 mm hypervascular nodule in the left adrenal consistent with small phaeochromocytoma.
This case illustrates challenges in diagnosing phaeochromocytomas. Surgical guidelines advocate surgery based on the concordance of the biochemical results which should be at least twice upper limit of normal along with confirmatory radiological imaging. This patient is clearly symptomatic with only borderline evidence of catecholamine access but imaging now suggesting left phaeochromocytoma. Should this man be offered surgery?

DOI: 10.1530/endoabs.34.P177

P178

Inhibition of radioiodine uptake by PBF in breast cells is consistent with sodium–iodide symporter repression in the thyroid

Vikki Louise Poole, Martin Read, Rachel Watkins, Bhavika Modasia, Gavin Ryan, Kristien Boelaert, Jayne Franklyn, Vicki Smith & Christopher McCabe
University of Birmingham, Birmingham, UK.

Whilst, radioiodine ablation is an effective therapy for many patients with thyroid cancer, a subset of patients are incapable of accumulating sufficient iodide-131 for effective treatment, due to low sodium–iodide symporter (NIS) activity. Previous work has identified that the overexpression of pituitary tumor transforming gene (PTTG) binding factor (PBF) in thyroid cells leads to the redistribution of NIS from the plasma membrane into intracellular vesicles, thereby reducing radioiodine uptake. With radioiodine being proposed as a potential treatment for breast carcinomas, where PBF has been reported to be overexpressed, it is important to discern the relationship between PBF and NIS in breast cancer cell lines. Immunofluorescent microscopy revealed co-localisation between NIS and PBF in co-transfected MCF7 and T47D cells, with increased intracellular staining for NIS compared to cells transfected with NIS alone. We have recently identified PBF as a tyrosine phosphorylated protein, with Src phosphorylation at residue Y174 critical to NIS regulation in thyroid cells. Importantly, phosphorylated PBF co-localised at the plasma membrane with NIS in T47D breast cells. In functional studies using iodine-125 in MCF7 and MDA-MB-231 cells, PBF significantly repressed radioiodine uptake in cells expressing exogenous NIS (25% and 30% reduction respectively, both $n=3$ and $P<0.05$). Treatment with PP1, a Src-inhibitor shown to inhibit the phosphorylation of PBF, restored the ability of MCF7 and MDA-MB-231 cells to uptake iodine-125 (1.24- and 1.69-fold increase respectively, $n=3$, $P<0.05$). PBF transfection also repressed radioiodine uptake in MCF7 cells treated with the NIS-inducing reagents all-trans retinoic acid (ATRA) and dexamethasone (31% reduction, both $n=3$ and $P<0.05$), with preliminary data suggesting that this reduction can also be overcome using PP1 treatment. Taken together, these data suggest that PBF can alter the subcellular location of NIS and thereby reduce the ability of breast carcinoma cell-lines to take up iodide, consistent with those findings previously reported in thyroid cells.

DOI: 10.1530/endoabs.34.P178

P179

Expression modulation of tumour necrosis factor α and tumour necrosis factor receptor 1 genes in breast cancer cell lines (MCF-7) by some selected indigenous cytotoxic plants

Titilola Aderonke Samuel, Babatunde James & Olubunmi Abiola Magbagbeola
University of Lagos, Lagos, Nigeria.

The use of herbal intervention in the treatment of tumors is widely spread in all regions of the developing world even though there are insufficient data on their possible molecular mechanism of action. Thirty percent of all cancers in women

occur in the breast making it the most commonly diagnosed female cancer. The cytotoxic properties of the 80% aqueous-ethanol crude, *n*-hexane, chloroform, ethylacetate, detannified, and tannin fractions of *Curculigo pilosa's* Rhizomes, *Icacina trichantha's* leaves, *Anthocleista djalonensis's* leaves, *Gladiolus psittacinus's* bulbs, *Tapinanthus bangwensis's* leaves, and *Spilanthes ficaulis's* leaves were evaluated for tumor necrosis factor α (TNFα) and TNF receptor (TNFR1) gene modulations. Following brineshrimp lethality assay, fractions of each plant (detannified *Icacina trichantha*, crude extract of *Curculigo pilosa*, hexane fraction of *Spilanthes ficaulis*, detannified *Anthocleista djalonensis*, hexane fraction of *Tapinanthus bangwensis* and crude extract of *Gladiolus psittacinus*) with the lowest LC_{50} were selected for the gene expression study. Concentrations that are fivefold lower than the LC_{50} of the six fractions were inoculated in triplicates into MCF-7 cells for 48 h, after which the expression of TNFα and TNFR1 genes were examined. TNFR1 gene expression was not observed in MCF-7 cell lines while TNFα expression was induced significantly ($P<0.05$) by the test fractions. In conclusion, the test fractions in this study do not induce apoptosis via the molecular mechanism of TNFα and TNFR1 expression but may support immunological activation due to the significant high levels of TNFα gene expression.

Reference

Thomas DB, Gao DL, Ray RM, Wang WW, Allison CJ *et al.* Randomized trial of breast self examination in Shanghai; final result. *J Natl Cancer Inst* 2002 **94** (19) 1445–1457.

DOI: 10.1530/endoabs.34.P179

P180

Characteristics of 'foregut' carcinoid tumours occurring in multiple endocrine neoplasia type 1

Lisa Yang, Kirsty Mann, Julian Winceslaus, Roaid Khan, Scott Akker, Mona Waterhouse, William Drake & Maralyn Druce
St Bartholomew's Hospital, London, UK.

Background

The glands most commonly affected in MEN1 are parathyroid, pituitary and pancreas. Pancreatic neuroendocrine tumours (pNETs) are of foregut origin, but a number of other so-called 'foregut carcinoid tumours' may also occur in MEN1, including tumours of bronchial, thymic, and gastrointestinal origin. Reported rates of prevalence of these latter tumours vary from 2% for thymic and bronchial carcinoids, to 10% for gastric carcinoids. Thymic carcinoids have been reported as aggressive in nature, whereas bronchial carcinoids are thought to be mostly indolent, with no effect on increased mortality in MEN1.

Aims

To evaluate the prevalence and characteristics of carcinoid tumours in current patients under follow-up with MEN1 at a tertiary referral centre.

Methods

Data were collected from case notes, histopathology reports, and cross-sectional imaging.

Results

Forty-six patients were under current follow-up with MEN1, 17 males and 29 females, mean age 53.7 years (range 18–79). Thirty-one foregut carcinoids were identified in 27 patients, of which 25 were pNETs. Six patients had non-pancreatic foregut carcinoids, five of which were intrathoracic (three bronchial, one thymic, and one indeterminate) and one was of gastric origin. Hyperparathyroidism was present in all six patients, four had a concurrent pNET (two gastrinomas and two non-secretory), and two had pituitary lesions. The three bronchial carcinoids included one successfully resected hormone-secreting tumour, and two non-secreting bronchial carcinoids, both of which showed malignant potential. One thymic carcinoid was successfully resected and the inderminate mediastinal mass is currently under investigation, but shows features of neuroendocrine carcinoma potentially of thymic origin.

Discussion

In this contemporary series of non-related MEN1 patients, the prevalence of foregut carcinoids was 59%, and of non-pancreatic foregut carcinoids 13% (predominantly bronchial), which is higher than in most reported series. In contrast to previous studies, non-secretory bronchial carcinoids showed aggressive characteristics in these patients. Current consensus guidelines suggest 1–2 yearly surveillance imaging with CT or MRI to detect intrathoracic carcinoids. Further prospective studies are required to guide decisions regarding prophylactic resection of asymptomatic pulmonary nodules.

DOI: 10.1530/endoabs.34.P180

P181

Inherited mutations in the SDH complex increase metastatic malignant potential of paragnglioma and phaeochromocytoma tumours

Bahram Jafar-Mohammadi[1], Louise Izatt[2], Klaus-Martin Schulte[3], Paul V Carroll[4], Barbara M McGowan[4], Jake K Powrie[4], Benjamin C Whitelaw[1], Debashis Sarker[5], Salvador Diaz-Cano[6] & Simon J B Aylwin[1]
[1]Department of Endocrinology, King's College Hospital, London, UK; [2]Department of Clinical Genetics, Guy's and St Thomas's Hospital, London, UK; [3]Department of Endocrine Surgery, King's College Hospital, London, UK; [4]Department of Endocrinology, Guy's and St Thomas's Hospital, London, UK; [5]Department of Medical Oncology, Guy's and St Thomas's Hospital, London, UK; [6]Department of Histopathology, King's College Hospital, London, UK.

Phaeochromocytomas (PCC) and paraganglioma (PGL) are neural crest tumours arising from the chromaffin producing cells of the adrenal medulla or sympathetic/parasympathetic system respectively. Recently, in part due to advances in high throughput sequencing, our understanding of the genetic predisposition to these tumours has greatly increased. To date, 13 genes have been implicated in the pathogenesis of these conditions (ten available for testing at our centre). Recent studies indicate that ~30–40% of patients with PCC/PGL may harbour a genetic predisposition to the condition. We aimed to determine the frequency that metastatic PCC/PGL was associated with mutations in known susceptibility genes.

The genetic profile of all individuals diagnosed with metastatic PCC and PGL in our centre was ascertained and compared to individuals with PCC/PGL without evidence of metastasis. Over the past 5 years 82 individuals with a diagnosis of PCC or PGL fulfilled the criteria for genetic testing. This included 16 individuals with metastatic disease. Among the patients with confirmed metastatic disease 13/16 (81%) had a genetic mutation identified in SDHB, SDHA, or SDHD predisposing to PCC and PGL. However, among those patients with no metastatic disease identified to date, only 42% (29/69) had a genetic mutation identified ($P=0.001$). Among the subjects with metastatic PCC, 11/13 had mutations (85%) in SDHB.

Our results imply that the identification of a mutation in the known PCC/PGL susceptibility genes confers an increased metastatic potential and also subjects with metastatic disease are most likely to harbour mutations in SDHB. Subjects with metastatic PGL/PCC are highly likely to have a genetic predisposition. In addition, identification of those individuals with PGL/PCC with a genetic mutation should be considered at high-risk for harbouring tumours with metastatic malignant potential.

DOI: 10.1530/endoabs.34.P181

P182

Pre-clinical assessment of the impact of Erlotinib on adrenocortical cancer cells proliferation

Dorota Dworakowska[1,2], Dorota Dudka[1,2], Gregory Weitsman[3], Peter King[4], Harshini Katugampola[4], Márta Korbonits[4], Klaus-Martin Schulte[1], Salvador Diaz-Cano[1], Alan McGregor[1], Ashley B Grossman[5], Simon Aylwin[1], Krzysztof Sworczak[2] & Tony Ng[3]
[1]Department of Endocrinology, Kings College Hospital, London, UK; [2]Department of Endocrinology and Internal Medicine, Medical University of Gdansk, Gdansk, Poland; [3]Richard Dimbleby Department of Cancer Research, Kings College London, London, UK; [4]Department of Endocrinology, Barts and the London Hospital, London, UK; [5]Oxford Centre for Endocrinology, Diabetes and Metabolism, Oxford, UK.

Introduction

Adrenocortical carcinoma (ACC) has a poor prognosis and limited therapeutic options. The epidermal growth factor receptor (EGFR) expression was found to be a good discriminator between malignant and benign adrenal tumours, but was mutated only in 3–10% of ACC cases.

Aim

The aim of this study was to assess the effect of inhibition of EGFR with targeted therapies, i.e. Erlotinib (with and without EGF stimulation) on ACC cell proliferation in a pre-clinical setting.

Material and methods

Proliferation of ACC cells (H295R cell line and primary adrenocortical tumour culture) was assessed by Alamar blue assay after 24, 48, and 72 h of incubation with inhibitor at presence or absence of EGF. The expression and activation/inhibition of EGFR, and downstream signalling (Akt, Erk1/2, and mTORC1) was detected by western blot analysis.

Results
Despite the fact that the expression of EGFR was below the detection level using western blotting, activation of downstream signalling by EGF was shown by activation of Erk1/2 and Akt proteins. Erlotinib decreased cell proliferation rate after 24, 48, and 72 h of treatment, and the effect was enhanced in the presence of EGF. These effects were observed both in H295R cell line and in primary tumour culture.
Conclusions
Erlotinib alone inhibits cell proliferation and acts more potently if used jointly with EGF. We hypothesize that this effect may be associated with the change in cell metabolism caused by EGF. Therapy with anti-EGFR agents may be successful in ACC patients but requires further study.

DOI: 10.1530/endoabs.34.P182

P183

Long-term negative effects in young cancer survivors: metabolic disorders in the patients after complex treatment for brain tumors and acute lymphoblastic leukemia in childhood and adolescence

Tatiana Tselovalnikova[1], Maria Pavlova[1], Alexey Zilov[1], Nadezhda Mazerkina[2] & Olga Zheludkova[3]
[1]Sechenov First Moscow State Medical University, Moscow, Russia; [2]Burdenko Neurosurgery Institute, Moscow, Russia; [3]Federal Scientific and Clinical Centre of Pediatric Hematology, Oncology and Immunology named after Dmitry Rogachev, Moscow, Russia.

Aim
The aim of this study was to examine the incidence of metabolic abnormalities in adults getting treatments of brain tumors (BT) and acute lymphoblastic leukemia (ALL) in their childhood or adolescence.
Methods
patients were divided in two groups. In group 1: 31 BT survivors (13 women and 18 men) were included. All patients received craniospinal irradiation up to 36 Gy and boost to the tumor up to 55 Gy and chemotherapy. The group 2: 18 ALL survivors (nine women and nine men). All of them received cranial irradiation in dose up to 18 Gy and chemotherapy. The median age was 20 (18; 22) and 20.5 (19; 23), age at the time of treatment – 12 (9; 15) and 7.5 (5; 9), follow-up period was 5 years (2; 10) and 13 years (12; 16) respectively. Metabolic profile was examined.
Results
In the 1st group: we registered elevation of LDL-c (>3.4 mmol/l) in seven patients, both LDL-c and triglycerides (TG) (>1.2 mmol/l) in six. Twelve of them had GH-deficiency, diagnosed with insulin tolerance test. OGTT was performed in 13 patients: FPG = 6.1 mmol/l was found in one patient. The median of BMI – 19 (17; 21.8). The BMI correlated with TG ($r=0.4$, $P=0.02$).
In the 2nd group: four patients had dyslipidemia: elevation of LDL-c ($n=2$) and TG ($n=2$). OGTT was performed in 12 patients, one woman had FPG = 6.2 mmol/l in combination with obesity (BMI = 49.1 kg/m2). Three patients had obesity. HOMA-index >2.5 was in seven patients. The age at the time of observation was negatively correlated with GH ($r=-0.723114$, $P=0.007872$). There were no significant differences in level of metabolic parameters between two groups.
Conclusions
BT survivors showed elevated level of LDL-c in 22.5%, increased of LDL-c and TG in 19.4%. In ALL survivors elevated level of LDL-c and TG was diagnosed in 11.1%. 16% ALL survivors had obesity. There were no abnormalities in carbohydrate metabolism.

DOI: 10.1530/endoabs.34.P183

P184

Diagnosis of adrenocortical carcinoma; urinary steroid profiles measured by GC:MS

Frances McManus[1], Jonathan Hannay[1], Patrick O'Dwyer[1], David Shapiro[2] & Marie Freel[1]
[1]Western Infirmary, Glasgow, UK; [2]Royal Infirmary, Glasgow, UK.

Introduction
Gas chromatography/mass spectrometry based steroid profiling has been proposed as a tool for differentiating adrenocortical carcinoma (ACC) from benign adrenocortical adenoma (ACA). We retrospectively examined urinary steroid profiles from ten patients with ACC and 14 patients with ACA to determine, if the steroid profiles were predictive in distinguishing benign from malignant disease.
Methods
Patients who had undergone adrenalectomy for resection of adrenal mass in the Western Infirmary, Glasgow were identified from theatre list and records of multidisciplinary team meetings between 2006 and 2013. Patients with preoperative urinary steroid profiles measured by GC:MS were included. Clinical details and histopathology was confirmed from case records and duration of follow-up was recorded.
Results
ACAs were identified, (of which nine were non-functioning and five were cortisol secreting) while ten ACCs were identified. Of the ACAs two were males and eight were females while in the ACC group there were four males and five females. There were no statistically significant differences in the urinary steroid profile when analysed as continuous variables although in general, median values of most compounds (with the exception of cortisol metabolites and 11-oxo-pregnanetriol) were higher in ACC patients. When analysed as a binary outcome (normal vs above normal for any compound), using any abnormality of the urinary steroid profile had a sensitivity of 90% but a specificity of only 42%. If only the most informative compounds were included (pregnenetriol, THA, and THS), the sensitivity remained at 90% and specificity rose to 92%.
Conclusions
Urinary steroid profiles may provide additional information to other clinical parameters in the work up of adrenal lesions in differentiating benign from malignant disease. This may be a useful tool in the future in assisting management decisions regarding surgical intervention as opposed to conservative treatment of this difficult condition.

DOI: 10.1530/endoabs.34.P184

P185

A rare adrenal lieomyosarcome in a subject with lupus and the antiphospholipid syndrome

I N Khan[1], A Guha[1], M A Adlan[1], M Stetchman[2] & L D Premawardhana[1,3]
[1]Section of Endocrinology, Ysbyty Ystrad Fawr Hospital, Hengeod, Wales CF82 7EP, UK; [2]Section of Endocrine Surgery University Hospital of wales Cardiff, University Hospital of Wales, Heath Park, Cardiff CF14 4XN, UK; [3]Section of Endocrinology, University Hospital of Wales, Cardiff, Wales, UK.

Introduction
Adrenal incidentalomas (AI) are mostly benign and non secretory, but some are malignant. Current diagnostic and management algorithms are not fail safe when assessing malignant potential, although functional status is much easier to assess. We present a subject with multiple comorbidities whose AI was a very rare adrenal leiomyosarcoma (AL)
Case presentation
A 40-year-old woman with SLE and the antiphospholid syndrome on lifelong warfarin therapy, presented with left sided loin pain. She was normotensive and did not have signs of endocrinopathy. Ultrasound scans showed scarring or an angiomyolipoma of the left kidney, but subsequent CT scans showed a 2.7× 3.9 cm abnormality in the left adrenal. MRI scans several months later confirmed this lesion, without significant fat and no interval growth. Further investigations confirmed cortisol after 1 mg dexamethasone, urinary free cortisol, urinary catecholamines and metanephrines, aldosterone:renin ratio, random 17-hydroxy-progesterone and chromogranin A and B were all within the reference range. Although, an adrenal bleed was thought likely initially in view of her warfarin therapy and acute presentation, she underwent adrenalectomy because of an increase in size of the lesion between scans. Histology confirmed the typical appearances of a leiomyosarcoma. She had subsequent radiotherapy for localised disease.
Discussion
Our subject presented with a rare AL. We like to highlight the following about her diagnosis and management – i) the initial diagnosis of an adrenal bleed in this warfarinised woman, had to be revised because of interval growth of the mass; ii) the tumour was relatively small compared to typical ALs described in the literature – average size of ~11 cm; and iii) this subject has survived for well over 48 months despite a malignant AL perhaps because of early detection and small localised tumour, resulting from her acute presentation.

DOI: 10.1530/endoabs.34.P185

P186

RET genetic screening in patients with multiple endocrine neoplasia type 2 and medullary thyroid carcinoma: experience of the Exeter Molecular Genetics Laboratory

Martina Owens[1], Bijay Vaidya[2] & Sian Ellard[1]
[1]Molecular Genetics, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK; [2]Diabetes and Endocrine Health, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK.

Introduction
Mutations in the *RET* gene cause multiple endocrine neoplasia type 2A (MEN2A) , MEN2B, and familial medullary thyroid carcinoma (FMTC). The identification of a germline *RET* mutation aids clinical management, enables the identification and predictive testing of at risk family members and provides reassurance for mutation-negative family members. In the research setting, mutations in exons 5, 8, 10, 11, 13–16 of the *RET* gene have been identified in >98% MEN2A and FMTC cases[1,2]. Approximately 95% of individuals with the MEN2B phenotype have the *RET* p.Met918Thr (p.M918T)[1] or p.Ala883Phe (p.A883F) mutation[3]. Approximately 7% patients with presumed sporadic medullary thyroid carcinoma have a germline mutation[4]. However, mutation detection rate in routine diagnostic setting is less known.
Methods
Our laboratory has been offering routine diagnostic *RET* testing for MEN2 and MTC for 16 years and have performed genetic screening in 720 cases (which included 269 cases of apparent sporadic MTC). We examined the cases we had tested in order to determine our mutation detection rate.
Results
We identified a *RET* mutation in 70 cases (10%) of our cohort: 16% of MEN2A cases, 30% of MEN2B cases, and 14% cases with FMTC. Further testing of the remaining exons of the *RET* gene in 21 patients did not detect any mutations. We identified a germline mutation in 9% of apparent sporadic MTC patients in our cohort.
Conclusions
Our data suggests that the mutation detection rate is lower in routine diagnostic laboratories than in a research setting, which may be due to less stringent testing criteria. Our data supports American (ATA) and European Thyroid Association (ETA) recommendation that all patients with MTC should be offered germline *RET* testing.

References
1. Eng *et al*. *JAMA* 1996 **276** 1575–1579.
2. Hansford and Mulligan. *J Med Genet* 2000 **37** 817–827.
3. Gimm *et al*. *J Clin Endocrinol Metab* 1997 **82** 3902–3904.
4. Romei *et al*. *Clin Endocrinol* 2011 **74** 241–247.

DOI: 10.1530/endoabs.34.P186

P187

MIBG-avidity in genetically distinct phaeochromocytoma and paraganglioma populations

Ross Jack[1], Robert Lindsay[3], Nicola Bradshaw[2], Marie Freel[3] & Colin Perry[4]
[1]Medical School, University of Glasgow, Glasgow, UK; [2]Department of Clinical Genetics, Southern General Hospital, Glasgow, UK; [3]BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK; [4]Department of Endocrinology, Western Infirmary, Glasgow, UK.

Phaeochromocytomas (PHAEOs) and extra-adrenal paragangliomas (PGLs) are rare neuroendocrine tumours. As many as 35% may have an identifiable germline mutation, most commonly in the genes encoding RET, VHL or subunits of succinate dehydrogenase (SDHx).
[^{123}I]-labelled metaiodobenzylguanidine (^{123}I-MIBG) scintigraphy is used to localise PHAEOs/PGLs, while ^{131}I-MIBG is used as therapy in malignant disease. Uptake of radioisotope may vary with genotype; ^{18}F-FDG–PET is more sensitive than MIBG in tumours associated with SDHB mutations. We compared MIBG avidity in genetically defined PHAEO/PGL patients with those without mutations in RET, VHL or SDHx.
We undertook a clinical record review of 55 patients with histologically proven PHAEOs/PGLs attending the Endocrinology Unit at the Western Infirmary, Glasgow.
Forty three of the 55 had genetic testing performed; in 31/43 (72%) a genetic mutation was identified. 18 (41.9%) had an SDHB mutation and 4 (9.3%) had an SDHD mutation; 6 (13.9%) had a mutation in VHL; 3 (7.0%) had a mutation in RET, and 12 (27.9%) had negative genetic testing.
Pre-operative MIBG scans were performed in 39 of 55 patients. 33 patients (84.6%) had MIBG avid tumours. 11/13 (84.6%) patients with an SDHB mutation were MIBG avid; 1/2 (50%) SDHD mutations were MIBG avid (total SDHx mutation group 12/15 (80%) MIBG avid). The other populations were as follows: RET 2/2 (100%); VHL 3/3 (100%); and sporadic 7/8 (87.5%) MIBG avid.
We found a higher than anticipated proportion of PHAEOs/PGLs with an identifiable germline mutation (56.4%). This may reflect the clinical service and the population it serves. MIBG avidity was no different in tumours associated with a genetic predisposition compared to those negative for RET, VHL, and SDHx mutations. We suggest that MIBG uptake is measured pre-operatively in PHAEOs/PGLs where otherwise indicated, irrespective of genotype.

DOI: 10.1530/endoabs.34.P187

P188

SDHB surveillance regime: a single UK institution experience

Umasuthan Srirangalingam, Fazia Khan, Kirun Gunganah, Anju Sahdev, Mona Waterhouse, Maralyn R Druce, William M Drake & Scott A Akker
St Bartholomew's Hospital, London, UK.

Background
Succinate dehydrogenase B (SDHB) associated disease has been characterised by the presence of extra-adrenal paragangliomas with a high rate of metastatic transformation. There is currently no consensus as to the appropriate surveillance regimes for these subjects. We present the surveillance data from a single UK institution with an SDHB surveillance regime which includes annual MR imaging of the abdomen, biennial imaging of the neck, thorax and pelvis and annual urinary/plasma metanephrines.
Results
The SDHB cohort includes 76 subjects. Six subjects were ruled out of the study to leave 70 subjects under surveillance. Thirty-three subjects developed a chromaffin tumour. Nine subjects (27% of those with tumours) had metastatic disease, four subjects (12% of those with tumours) had multiple tumours and six subjects were deceased. Of those subjects with tumours 70% (23/33) had abdominal disease, 15% (5/33) had pelvic paragangliomas, and 9% each (3/33) had head/neck and thoracic paragangliomas. Three subjects had renal cell carcinomas. Seven subjects (10% of all SDHB subjects) have had tumours identified on subsequent surveillance imaging which include four subjects with chromaffin tumours, two subject with a renal cell carcinoma (one also having an additional transitional cell carcinoma of the bladder) and one subject with a papillary thyroid carcinoma. One of the chromaffin tumour identified included a 3 mm bladder paraganglioma which was successfully excised. Four further subjects have small lesions identified and being tracked with diffusion weighted MR imaging, potentially suspicious for chromaffin tumours but yet to be confirmed.
Discussion
We advocate an intensive surveillance regime, predominantly focused on the abdomen, in subject with SDHB mutations given that the majority of disease is centred in the abdomen, 10% of these subjects will be found to have disease on subsequent scanning (both chromaffin and non-chromaffin tumours) and approximately a third of those with tumours will develop metastatic disease.

DOI: 10.1530/endoabs.34.P188

P189

Multiple endocrine neoplasia type 2A in a large family with a C620G mutation of the RET proto-oncogene: diagnostic, treatment, and ethical challenges

Rajeev Raghavan, Venkata Katreddy, L Leiw, A Garnham, R Gama, P Strouhal, H Buch, A Viswanath & BM Singh
Wolverhampton Diabetes Centre, Wolvehampton, UK.

Introduction
Multiple endocrine neoplasia type 2 (MEN2) is an autosomal dominant multi-glandular tumour syndrome, caused by RET germline mutations.We present a family with three affected generations identified by predictive testing.
Index case and cohort details
A 40-year-old lady referred to ENT clinic with a swelling in the neck. Ultrasound confirmed multinodular goitre with FNAC-THY3. Diagnostic hemithyroidectomy was followed by total thyroidectomy for medullary thyroid cancer (MTC). Genetic testing confirmed heterozygous missense mutation (c.1858T>G, P.Cys620Gly; exon 10 of RET gene).Family history, pedigree charting, and predictive testing identified three affected generations. First generation: mother

had chemodectoma and uncle had MTC. Second generation: three out of seven siblings (6F and 1M) of the index case affected (details in table below). Third generation: one offspring has confirmed C-cell hyperplasia following thyroidectomy.

Discussion

The management of this cohort posed complex ethical, diagnostic, and management challenges. As an example with sibling-1, investigations revealed elevated calcitonin, normal urine catecholamines but slightly elevated plasma metanephrines. CT adrenal was normal, but MIBG scan showed increased uptake in the left adrenal. There was non-acceptance of diagnosis and indecisiveness regarding proposed interventions. Following MDT discussion and concerted efforts to engage and prepare the patient, unilateral adrenalectomy was undertaken before thyroidectomy. Plasma metanephrines normalised post adrenalectomy. Subsequently when the date for total thyroidectomy was imminent, an unplanned pregnancy was confirmed. At this point following further extensive discussion of options (in the context of no nodules/cervical lymphadenopathy and calcitonin mildly elevated and stable over 18 months) the patient elected to continue pregnancy with plan for *post-partum* thyroidectomy.

Conclusion

Our cohort illustrates the challenges posed in managing this rare condition from identifying the mutation, disseminating the diagnosis and communicating management options to affected individuals. Expected behaviour characteristics could vary significantly from that predicated on genotype:phenotype correlations quoted in the literature.

Characteristics of 2nd generation	Index case	Sibling 1	Sibling 2	Sibling 3
Age	43, F	42, F	47, F	40, F
MTC	Yes	Yes	Yes	Yes
Thyroidectomy	Hemi followed by total	Awaiting	Total	Total
Pheochromocytoma	No	Yes	Bilateral large	No
Adrenalectomy	No	Left total	Bilateral total	No
Parathyroid axis	Normal	Normal	Normal	Not known
Mutation in offspring	0/3	1/3	0/3	0/3

DOI: 10.1530/endoabs.34.P189

P190

An incidental diagnosis…

Irfan Khan & Paul Lambert
Musgrove Park Hospital, Taunton, Somerset, UK.

MEN2a is a rare genetic endocrine disorder associated with mutation of RET oncogene on chromosome 10. It involves lesions in thyroid, parathyroid and adrenal glands. Medullary thyroid carcinoma is the pathological hallmark and usually the first presentation of this syndrome.

Clinical case

A 64-year-old lady with previous history of hypertension, IHD, and hypercholesterolemia was referred to Endocrine clinic after she was incidentally found to have high calcium (3.2) and inappropriately high PTH and was diagnosed as having primary hyperparathyroidism. There was no previous history of any endocrine problems and her BP was well controlled with Atenolol and Lisinopril. Neck ultrasound and SISTAMIBI scan demonstrated a single parathyroid adenoma and she was referred for parathyroidectomy. Intraoperatively the surgeon noticed a firm suspicious looking nodule in the thyroid and took a FNA sample from that nodule which was later reported a Thy3 follicular lesion. The case was discussed in thyroid surgery/Endocrine MDT and it was decided to perform a right hemithyroidectomy. Histopathology showed that the large palpable nodule was actually benign but it incidentally showed a 1 mm papillary carcinoma and 1.5 mm medullary carcinoma. The presence of primary hyperparathyroidism and medullary thyroid carcinoma raised the possibility of MEN2a. Genetic testing and calcitonin levels are awaited. Urinary metanephrine levels (checked twice) are normaI.

If RET oncogene is positive, this would make an atypical case of MEN2a because of the late presentation (usually presents in third decade) and atypical presentation (usually the first presentation is with medullary thyroid carcinoma followed by phaeochromocytoma).

DOI: 10.1530/endoabs.34.P190

P191

ATR kinase function is modulated by the proto-oncogene PBF

Hui Fan[1], Teresa Gagliano[2], Martin L Read[1], Jayne A Franklyn[1], Kristien Boelaert[1], Andrew S Turnell[1] & Christofer J McCabe[1]
[1]University of Birmingham, Birmingham, UK; [2]University of Ferrara, Ferrara, Italy.

Disruption of DNA damage response pathways results in an accumulation of genetic mutations, gene amplifications and chromosome alterations, which are key initiating factors in cellular transformation and oncogenesis. PBF is a multifunctional proto-oncogene which is overexpressed in thyroid, pituitary and breast cancers, with roles in cell transformation, invasion and transporter regulation. Recently, we reported that targeted transgenic expression of PBF promotes genetic instability (GI) *in vivo*. Now, we provide evidence of a direct functional interaction between PBF and the single strand DNA repair (SSDR) enzyme Ataxia telangiectasia and RAD3-related protein (ATR). In HeLa cells, PBF significantly attenuated the ATR-mediated phosphorylation of downstream DNA repair targets Chk1 and RPA32 in response to u.v. treatment. By contrast, PBF was not involved in the double-stranded break repair (DSBR) response to ionising radiation, with the phosphorylation of the key DSBR proteins γH2AX, NBS1, SMC1 and Chk2 unaffected by PBF levels in response to 10 Gy of IR. We thus confined our studies to SSDR. PBF protein expression was stabilised by increasing doses of u.v. from 20 to 130 J/m^2, and with increasing time (0, 0.5, 1, 2, 3, 4, and 6 h) following 20 J/m^2 u.v. treatment. Consistent findings were apparent in TT cells. FACS analysis demonstrated that u.v.-irradiation was associated with significantly increased numbers of HeLa cells entering mitosis in cells overexpressing PBF compared to vector-only control cells (0.8 vs 1.2%, $P<0.005$, $n=5$). Given that PBF induction in response to single strand DNA damage resulted in altered ATR function, we investigated whether PBF and ATR interact *in vitro*. Forward and reverse co-immunoprecipitation assays confirmed specific interaction between ATR and PBF in HeLa cells, although the stringency of binding did not increase over time (0, 0.5, 1, 3, and 6 h) following u.v. induction of single strand breaks. Taken together, our data demonstrate a novel potential mechanism to explain our finding that PBF induces GI *in vivo*; PBF binds to the single strand DNA repair enzyme ATR, dysregulating its kinase activity and decreasing the phosphorylation of RPA and Chk1 following DNA damage. Oncogenic expression of PBF in endocrine cancers would thus confer a survival and proliferative advantage following DNA damage, facilitating neoplastic growth and tumourigenesis.

DOI: 10.1530/endoabs.34.P191

Nursing practise

P192

Steroid group education: developing a curriculum ensures good nursing practice is maintained

Jean Munday
Queen Alexandra Hospital, Portsmouth, UK.

Background

We have used group education sessions to instruct patients about steroid sick day rules for over 3 years. The groups were initially run by one Endocrine Nurse Specialist (ENS) so every group followed the same format with the same take home messages. With the development of a second ENS post there became an opportunity to share running the sessions. However, it was vital to ensure patients attending the groups were all given the same teaching regardless of who was running the group on a particular day. Therefore, a curriculum was developed to assist the newer ENS to run the groups effectively and to ensure good practice was maintained.

Innovation

A curriculum was developed which encompassed all aspects of preparing for, setting up and running a teaching session for patients on steroid replacement. The ethos of encouraging patients to share their stories, and problem solve scenarios was outlined in the document.

Conclusion

Having a written curriculum has helped the ENSs to maintain consistency during their steroid group education sessions. It was also a point of reference to assist the newer ENS when she first started to run groups independently. More recently an ENS from another centre has visited us to observe a session and we were able to provide her with the curriculum document to take back and use in her hospital.

DOI: 10.1530/endoabs.34.P192

P193

Time taken for GH-treated adolescent patients, transitioning to adult services, to reach IGF1 levels within the upper normal range: do we need to monitor more frequently?

Kerrie Grounds, Mohammed Didi, Pamela Corlett, Pauline Whittingham & Aftab Ahmad
Royal Liverpool University Hopital, Liverpool, UK.

Introduction
According to NICE: adults receiving GH treatment, IGF1 levels should reach therapeutic range by 9 months. Patients are reviewed at 1, 3, 6, 9, 12 months and 6 months thereafter in our clinics. GH dose is titrated by 0.1 mg each visit. This work aims to compare the time it takes to get the IGF1 in range, between adults and adolescent patients attending our specialist regional clinics.
Method
We performed a retrospective audit of 20 patients with GH deficiency (ten attended Transitional clinic: men = 6, age 18–26 years, and ten attended the adult GH clinic: men = 10, age 41–75 years. Data were gathered using patient case notes and hospital systems).
Results
Of the ten adolescent patients, 20% achieved the therapeutic IGF1 range within 9 months (10% at 3 months and 10% at 9 months). 80% achieved IGF1 target outside the 9-month period (10% at 12 months, 10% at 15 months, 30% at 21 months, 30% are still yet to reach their therapeutic range).
Of the ten adult patients, 70% achieved therapeutic IGF1 target within 9 months (20% at 1 month, 10% at 3 months, 30% at 6 months and 10% at 9 months). 30% achieved therapeutic IGF1 levels outside the 9-month period (10% at 12 months and 20% at 18 months).
The mean maintenance dose of GH for the adolescent patients was 0.6 mg and for the adult patients 0.2 mg.
Conclusion
Our results suggest adolescents do not reach therapeutic IGF1 as quickly as adults using GH replacement, most likely due to the higher GH dose requirements, which takes longer to achieve at 0.1 mg increments at 3-month follow-up intervals. We suggest either increasing the follow-up frequency or GH dose increments of 0.2 mg at current follow-up interval.

DOI: 10.1530/endoabs.34.P193

P194

Life, death and modern technology: an update

Fiona Anthonypillai
St George's Hospital NHS Trust, London, UK.

Data presented in 2011 identified a lack of steroid management knowledge in both patients and hospital staff. Numerous strategies have now been put in place to address this. These strategies have not only focused on patient education, but also on raising steroid awareness across the Trust.
The first 48 respondents of a pre steroid education nurse survey, concerning steroid awareness, showed that, 48% did not know what an adrenal crisis is, but that 65% knew that stopping steroids could lead to a deterioration in the patients' health. Regular nurse training sessions have been set up to teach nurses how to correctly manage 'steroid dependant' Inpatients and how to deal with an addisonion crisis. Each nurse is issued with a plastic 'endocrine prompt' card which identifies keys steps and pertinent questions to help general nurses care for endocrine patients. To alert staff to steroid dependence, steroid warning labels and stamps have been produced for case notes and a steroid management protocol has been written. The steroid pack, previously produced, has been made into a QR code for downloading to smart phones, addressing the 'on the go' requirement without being internet reliant or platform specific. To support patients in administering the emergency hydrocortisone injection, a 'step by step' patient video accessible from the trust web site has also been produced.
An audit of 120 patients educated through the 'steroid pack' showed that 87% of patients now know how to titrate their steroids in given scenarios and for those with Internet access, 86% found the patient video useful.
Steroid education and management for patients and staff alike must involve tools of reference accessible whenever required, so to help deal with the life threatening situation of cortisol insufficiency both within the hospital setting and outside.

DOI: 10.1530/endoabs.34.P194

P195

Intervention of ward visits by an endocrine nurse specialist and a protocol and in the management of hyponatraemia

Rhianne Mason[1], Charlotte Forbes[1], Seren Williams[1], Timothy McDonald[2] & Antonia Brooke[1]
[1]Department of Endocrinology, Royal Devon and Exeter Hospital, Exeter, UK; [2]Department of Biochemistry, Royal Devon and Exeter Hospital, Exeter, UK.

Hyponatraemia is associated with an increase in morbidity and mortality, prolonged hospital stays and poor assessment and management. Two audits were performed looking at acute medical unit (AMU) admissions with sodium < 130 mmol/l, before and after the introduction of a protocol and regular endocrine nurse specialist ward visits, 3 months apart (58 patients in April and 99 in July 2013).
Hyponatraemic patients were older than the AMU population (> 80 years: April 50%, July 46%, AMU 32%) and the length of stay was longer (9.12 days (D) (range 1–44) April (A); 10.57D (1–69) July (J); AMU 6.47D). The main four discharge specialties were Health Care For Older People, Endocrinology, Respiratory and Acute Medicine.
After the protocol, more patients with sodium < 127 mmol/l had improvement at 24 h (April 65% were better, July 82%) and sodium was more frequently normal at discharge (19%A vs 28%J). Serum and urine osmolalities, TSH and cortisol were more frequently measured.
Monitoring for all patients with sodium < 130 mmol/l was improved. Of the 41 notes examined in April and 74 in July, there were more fluid charts completed (49%A vs 64%J). Hyponatraemia was mentioned more frequently in the post take ward round (48%A vs 68%J) and fluid restriction more common (24%A vs 57%J). Assessment of fluid intake (43%A vs 20%J) and urinary sodium (19%A vs 22%J) remained poor, although not clinically relevant in all.
Only 19/58 patients had HRG codes for hyponatraemia in April and 26/99 in July (38/80 with Na < 127 mmol/l). The additional income over 2 months for correct coding would have been £6540 (equivalent to £39240 per year).
Introduction of a protocol and endocrine specialist nurse involvement improves assessment of hyponatraemia, although urine sodium and assessment of fluid intake remains poor. These interventions highlight the issue of hyponatraemia at an early stage, leading to a focus on interventional management.

DOI: 10.1530/endoabs.34.P195

P196

Treating vitamin D deficiency in coeliac patients

Nicci Komlosy & Caroline Jagger
Manchester Royal Infirmary, Greater Manchester, UK.

Vitamin D deficiency has been estimated to affect one billion people world wide[1]. It is especially prevalent in Coeliac disease which is estimated to affect one in a 100 people. The intolerance to absorb gluten reduces not only the absorption of calcium, but can also further induce secondary hyperparathyroidism, infertility and a small increased risk of bowel carcinoma and osteoporosis.
A joint nurse/dietetic Coeliac and Osteoporosis clinic was set up at Manchester Royal Infirmary in 2001 due to an increased demand of Coeliac patients which required specialist treatment. Patients are seen after confirmation of Coeliac disease through biopsy. The clinic operates every Friday morning with approximately we see approximately ten patients each week. The patients are seen by both specialists in clinic when then are reviewed.
A cohort of 50 Coeliac patients were tested for vitamin D deficiency at their routine appointment. Forty patients were found to be deficient in varying ranges. Twenty were treated with 40 000 IU colecalciferol daily for 10 days and the remainder where prescribed 20 000 IU to be taken weekly for 12 weeks.
The patients bloods were rechecked after 12 weeks, to ensure that they were vitamin D replete, the results suggested that there was no significant difference between loading the patients with the 10-day regime as opposed to the 12-week regime.
Incidentally most patients preferred the 40 000 IU over 10-day regime as they were less likely to forget to take it and reported a positive health benefit such as reduced bone pain and lethargy.
Reference
1. Holick M 2007.

DOI: 10.1530/endoabs.34.P196

P197

Integrating a new drug into clinical practice and enhancing the patient experience

Caroline Jagger
Manchester Royal Infirmary, Greater Manchester, UK.

Denosumab is a fully human MAB that inhibits bone resorption by neutralizing RANKL and was licensed in 2010 for the treatment of post-menopausal osteoporosis. It was first integrated in a northwest trust, under the care of the metabolic and bone disease department, as a second line treatment.
Since 2012 denosumab has been used in the outpatient clinic under a shared care agreement with the greater Manchester medicines management group, this agreement ensured patients were catered for the first 12 months in the hospital environment and then followed up in the community.
The administration of denosumab 60 mg/1 ml is a 6 monthly s.c. injection uses a prefilled syringe, it is stored in a refrigerator and once removed can be stored at room temperature for 28 days.
The experience of integrating a new drug into practice has implications for clinic capacity, and the patients. The patients have found it to be a positive experience as they can contact the helpline number to arrange a mutually convenient time to attend. This in turn reduces the waiting time in clinic. It also allows patients that were previously unsuitable for some osteoporosis treatments due to allergies or a reduced renal function to have the drug. The drug can be given quickly and as anaphylaxis is not a side effect of the drug patients do not have to wait after the injection and there is no fasting regimes patients only have to attend clinic twice for the injection and can have the rest of their treatments at their local general practice.
The benefit for practice is autonomy for the nurse, the patient experience is enhanced and a therapeutic relationship is formed built upon trust using the best available evidence, and concordance and compliance is increased due to decreased side effects.

DOI: 10.1530/endoabs.34.P197

P198

Emergency hydrocortisone kits: assessment of knowledge and skills of patients and family

Lisa Shepherd, Sadaf Ulnasah, Asad Rahim, Jayadave Shakher & Andrew Bates
Heart of England NHS Foundation Trust, Birmingham, UK.

It is recommended that patients with adrenal insufficiency should be in possession of an emergency hydrocortisone kit and have sufficient knowledge and skills to administer it. Historically, in our unit patient education was dependent on referral from Doctors to endocrine specialist nurses (ESN's). This study sought to assess if patients owned a kit and if patients and family were competent to administer the injection if required.
A 'snapshot' consecutive sample of primary and secondary adrenal insufficient patients, were audited using a quantitative questionnaire. All patients ($n=34$), who attended group education sessions in the endocrine outpatient department run by endocrine specialist nurses, for a period of 6 months in 2011–2012 were surveyed.
Of those audited 38% of patients possessed an emergency hydrocortisone kit. Although 41% had received previous training on the preparation of the hydrocortisone injection, no one had ever administered the injection themselves. Only 18% of family members had received training. 82% of patients said they wished to receive training on the preparation and administration of the injection compared to 68% of family. Almost half (44%) of patients, thought that their family knew when it was appropriate to administer an emergency injection. Worryingly, only 76% of patients carried a 'blue' steroid card and 35% wore medical alert jewellery.
Findings identified the number of patients who owned a kit needed to be dramatically improved. Education, training and support also required enhanced structure. Following changes recommended a universal approach to patient education has been adopted. Referrals of all adrenal insufficient patients are now made to the ESN's, to ensure all patients are educated and have access to emergency hydrocortisone kits. The outcome of the re-audit of this patient group has been submitted as a separate abstract and has demonstrated the positive advantage of nurse led education sessions.

DOI: 10.1530/endoabs.34.P198

Obesity, diabetes, metabolism and cardiovascular

P199

The effect of L-cysteine on appetite in humans

Anjali Amin, Amy Agahi, Niamh Martin, Mohamed Ghatei, Steve Bloom & Kevin Murphy
Imperial College London, London, UK.

High protein diets suppress appetite and facilitate weight loss, but are difficult to adhere to. Understanding the mechanisms by which protein suppresses appetite may establish targets for more acceptable interventions to treat or prevent obesity. Of particular interest is the concept of functional foods or novel products, which aim to potentiate satiety.
Receptor systems that respond to amino acids have been identified. However, the specific mechanisms regulating protein-induced satiety are unknown. Previous work has investigated the effect of specific amino acids which act as ligands for the following G-protein coupled receptors: CaR, T1R1/T1R3 and GPRC6A on food intake in rodents.
L-cysteine activates the CaR, the T1R1/T1R3 and the GPRC6A. A diet that includes high levels of whey protein, which contains high levels of L-cysteine, has been reported to be more satiating, and to suppress circulating levels of the orexigenic hormone ghrelin to a greater extent, than other types of protein in humans. Pilot studies suggested that ligands for the GPRC6A receptor can reduce food intake in rodents, and that this effect is at least partly mediated by a reduction in circulating ghrelin levels.
The role of L-cysteine in food intake in humans was thus investigated. Following an overnight fast, healthy volunteers were given either oral L-cysteine (0.04 or 0.07 g/kg), glycine (0.04 or 0.07 g/kg) or vehicle control in a double-blinded randomised manner. Following administration of the amino acid or control, visual analogue scales were completed and gut hormone analysis carried out every 15 min over a 2.5-h period. Results showed that oral administration of L-cysteine significantly reduced feelings of hunger ($P<0.05$) and reduced circulating ghrelin levels ($P<0.05$) compared to vehicle or glycine controls. These data suggest that L-cysteine may reduce appetite, and this effect may be mediated by a reduction in ghrelin.

DOI: 10.1530/endoabs.34.P199

P200

Analysis of serum FTO level and related factors in obese and T2DM patients

Jianjin Guo[1], Wei Ren[2,4], Jie Liu[2] & Weiping Jia[3,4]
[1]Department of Endocrinology, Second Affiliated Hospital of Shanxi Medical University, Taiyuan, China; [2]Shanxi Province People's Hospital, Taiyuan, China; [3]Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated Sixth People's Hospital, Shanghai, China; [4]Shanghai Diabetes Institute, Shanghai, China.

Background and aims
The global epidemic of obesity represents a severe threat to public health and quality of life. Fat mass and obesity-associated (FTO) gene was the first locus unequivocally associated with adiposity. The polymorphisms of FTO gene association with obesity and T2DM have been identified in large multiple populations, however, the correlation between FTO level and clinical parameters in is not completely understood. Here we analyze the relations of serum FTO to adiposity and glucose metabolism in obesity and T2DM individuals.
Materials and methods
The experiment designs the groups as fellow: subjects with normal glucose regulation and normal weight (NW–NGR, $n=96$), abdominal obese subjects with normal glucose regulation (OB-NGR, $n=67$), newly-diagnosed type 2 diabetes mellitus (T2DM) and its subgroups of T2DM with normal weight (NW-T2DM, $n=153$) and T2DM with abdominal obesity (OB-T2DM $n=116$). Serum FTO was detected by double antibody sandwich ELISA in fasting status. The levels of glucose, lipids, insulin and anthropometrical parameters such as BMI, fat content, waist circumference (WC) and waist hip ratio (WHR) were measured. Insulin sensitivity was assessed by HOMA-IR. Correlation analysis was performed in order to investigate the relationship between FTO and the detected parameters.
Results
After age and sex adjustment, serum FTO levels in OB-NGR, NW-T2DM, and OB-T2DM were significantly higher than that of NW-NGR (32.74 ± 2.65, 28.34 ± 1.96, 43.22 ± 3.48 vs 23.66 ± 1.62 pg/ml, $P<0.05$). There was no difference in serum FTO levels between OB-NGR and NW-T2DM, but the serum FTO levels in OB-T2DM was significantly higher than that of NW-T2DM ($P<0.01$) and OB-NGR ($P<0.05$). In the linear correlation analysis, serum FTO were positively correlated with BMI, WC, Fat%, WHR, TG, TC (P all <0.01); and also positively correlated with FINS, HOMA-IR, LDL-C, FPG (P all <0.05);

negatively correlated with HDL-C ($P<0.05$). Multiple stepwise regression analysis revealed BMI, WC, WHR, TG were the significant impact factor influencing the of the serum FTO level.
Conclusion
Serum FTO is not only strongly related to abdominal obesity and insulin resistance, but also associated with blood glucose and lipids. BMI, WC, WHR and TG were most significant independent determinants for serum FTO concentration. As such, the serum FTO levels would be a candidate metabolic marker obesity, especially abdominal obesity.
Key Words: FTO, T2DM, obesity, glucose, BMI
DOI: 10.1530/endoabs.34.P200

P201

Associations between regional body fat distribution and homocysteine levels in type 2 diabetes with coronary atherosclerosis patients
Wei Ren[1,2], Jianjin Guo[3] & Jie Liu[1]
[1]Shanxi Province People's Hospital, Taiyuan, China; [2]Shanghai Diabetes Institute, Shanghai, China; [3]Department of Endocrinology, Second Affiliated Hospital of Shanxi Medical University, Taiyuan, China.

Objective
To study the relationship between regional body fat distribution and homocysteine levels (Hcy) in type 2 diabetes (T2DM) with coronary atherosclerotic lesions and homocysteine (Hcy).
Methods
115 patients in department of endocrinology and cardiology of Shanxi Province People's Hospital from October, 2010 to May, 2011 was divided into T2DM with coronary atherosclerosis group 53 patients, T2DM with non-coronary atherosclerosis group 62 patients according to 64 row helical CT coronary imaging and oral glucose tolerance test. Sixty cases of healthy persons setted to the normal control group in our health medical center. There was no statistical difference in age and sex between the three groups by Student's *t*-test. Measuring fat mass/height^2, android/gynoid ratio, trunk leg fat ratio, trunk body fat %, trunk limb fat ratio; Hcy, glycated hemoglobin, fasting insulin, microalbuminuria/creatinine ratio, triglycerides, LDL, HDL, c-reactive protein; metabolic indices and counting insulin resistance index (HOMA-IR); BMI, waist–hip ratio (WHR). Comparing the difference of biochemical index and body fat measurement index in three groups doing the correlation analysis between T2DM with coronary atherosclerotic lesions and Hcy, the correlation analysis between Hcy and body fat index in T2DM with coronary atherosclerosis group.
Results
HOMA-IR, LnHcy have a statistics difference ($P<0.05$), multiple comparison have a statistics difference ($P<0.05$) in three groups. Logistic regression analysis shows Hcy is the independent risk factor of T2DM with coronary atherosclerotic lesions. Hcy level of triple vessel lesion group obviously higher than other groups ($P<0.05$). HOMA-IR, Hcy is independent risk factors of T2DM with coronary atherosclerotic lesions. android:gynoid ratio, trunk leg fat ratio, trunk body fat %, trunk limb fat ratio of T2DM with coronary atherosclerosis group are significantly higher than the rest groups ($P<0.05$). The four indexes are positively correlated with Hcy level, correlation coefficient is respectively ($r=0.640$, 0.452, 0.377, 0.323).
Conclusions
Hcy levels are obviously relevant with diabetes cardiovascular disease, Hcy is the independent risk factor of T2DM with coronary atherosclerotic lesions. T2DM with coronary atherosclerosis patients present centripetal fat distribution mode. Hcy is positively related with android/gynoid ratio, trunk leg fat ratio, trunk body fat %, trunk limb fat ratio.
Key Words: type 2 diabetes, diabetic trunk angiopathy, body fat distribution, homocysteine
DOI: 10.1530/endoabs.34.P201

P202

An opinion survey in Eastern Ghana: health behaviours and knowledge of diabetes in diagnosed diabetes mellitus type 2 patients
Gemma Buston
University of Birmingham, Birmingham, UK.

Introduction
As the countries in Sub-Saharan Africa become more developed, death from communicable diseases is decreasing, whilst a diagnosis of a non-communicable disease (NCD) such as diabetes mellitus type 2 (DM2) is becoming more commonplace. This opinion survey at Asesewa Government Hospital (AGH), Eastern Ghana, aimed to assess knowledge of risk factors and complications of DM2, and attitudes towards DM2, in patients diagnosed with the disease.
Methodology
A cross-sectional questionnaire was administered to DM2 patients in an opportunistic manner in outpatients clinics at AGH from 20 to 31 May 2013.
Results
A convenience sample of 21 patients was obtained. Prior to diagnosis, 2 (9.5%) were told that they were at risk of DM2. Following diagnosis, 21 (100%) claimed to have attempted a healthier diet, while 14 (66.7%) claimed to exercise at least three times per week. Knowledge of risk factors varied: 20 (95.2%) correctly acknowledged hypertension and family history, while 16 (76.2%) incorrectly identified unclean water as a risk factor. 21 (100%) correctly identified 'tiredness' as a symptom, with 1 (4.8%) incorrectly identifying 'swollen glands'. 20 (95.2%) recognised 'heart problems' as a complication, while only 1 (4.8%) correctly identified 'nerve problems'.
Conclusion
While knowledge of symptoms of DM2 was good, knowledge of risk factors and complications of the disease was limited. This author recommends a region-wide service evaluation of: i) education regarding healthy behaviours, ii) information provided post-diagnosis, iii) health promotion regarding when to seek help with respect to DM2.
DOI: 10.1530/endoabs.34.P202

P203

Effects of transdermally delivered insulin on some selected metabolic parameters of streptozotocin-induced diabetic male Sprague–Dawley rats
Silindile I Hadebe, Phikelelani S Ngubane, Metse R Serumula & Cephas T Musabayane
University of KwaZulu-Natal, Durban, KwaZulu-Natal, South Africa.

The tight glycaemic control in type 1 diabetes which requires uncomfortable multiple insulin injections is associated with patients' non-compliance. Therefore, methods which can sustain therapeutic controlled insulin release into the blood based on topical applications may be beneficial with glycaemic control. Accordingly, the present study investigated whether topically applied pectin insulin (PI) amidated matrix patch sustains insulin release into the bloodstream and control some selected deranged metabolic parameters in experimental diabetes. PI patches were prepared by dissolving pectin/insulin in deionised water with subsequent solidification with $CaCl_2$. Oral glucose tolerance (OGT) responses were evaluated in groups of streptozotocin (STZ)-induced diabetic rats given a glucose load (0.86 g/kg) after an 18 h fast followed by topical application of PI patches containing various insulin doses (2.47, 3.99, 9.57, and 16.80 μg/kg). Short-term (5 weeks) effects were assessed in animals applied thrice daily with topical PI (16.80 μg/kg) 8 h apart. Animals treated with drug-free pectin and insulin (175 μg/kg, s.c.) acted as untreated and treated positive controls, respectively. Blood samples and tissue samples were collected for the measurement of selected biochemical parameters and effects on the expression of insulin-stimulated enzymes and facilitative glucose transporters. OGT responses to PI patches exhibited lower blood glucose levels compared to untreated animals. Plasma insulin concentrations increased significantly following PI patch application with the highest dose eliciting the highest insulin levels by comparison with the lowest dose (4.52 ± 0.27 vs 7.13 ± 0.09 ng/ml). The transdermal PI treatment restored the reduced glycogen concentrations, expression of insulin-stimulated enzymes and facilitative glucose transporters in muscle and hepatic tissues observed in diabetic animals to near normalcy after 5 weeks. We suggest that transdermal PI delivers insulin into the bloodstream with concomitant amelioration of some metabolic parameters suggesting that the formulation may free diabetic patients from multiple insulin injections thereby improving patient compliance.
DOI: 10.1530/endoabs.34.P203

P204

Early insulin therapy: a new treatment approach for type-2 diabetes mellitus with HbA1c>7%
Harshitbhai Patel
Sardar Patel, Vallabh Vidyanagar, Gujarat, India.

Diabetes is a manageable but serious illness characterized by high blood sugar. In present study, comparison of treatment of metformin, insulin (biphasic isophane

insulin) and insulin (biphasic isophane insulin) + metformin in the patients of type 2 diabetes mellitus with HbA1c > 7% was performed. A total 45 patients of type 2 diabetes with HbA1c > 7% were selected. The selected 45 patients were divided into three groups of 15 each. First group was treated with metformin (1000 mg), second group was treated with insulin (10 IU) and third group was treated with insulin (10 IU) + metformin (500 mg). In the present study an attempt has been made to find out correlation of insulin, metformin and insulin + metformin therapy with various parameters like, fasting blood glucose level, postprandial blood glucose level and HbA1c from the data of the patients under study. Duration of treatment was 3 months. Fasting blood sugar (FBS), postprandial blood sugar (PP2BS) and HbA1c level were measured before treatment and after 3 months of each treatment course. Result of this study indicated that significant reduction in fasting and postprandial blood glucose level was observed in each treatment group. In all treatment groups, significant reduction in HbA1c level was also observed. Insulin + metformin therapy reduced HbA1c level below 7% while individual insulin and oral therapy (metformin) reduced HbA1c near 7%. It is concluded that insulin (biphasic isophane insulin) + metformin therapy is more effective in the treatment of type 2 diabetes mellitus with HbA1c > 7%. This study provides scientific evidence that insulin therapy in the treatment of type 2 diabetes can be used as first assault rather than last resort.

DOI: 10.1530/endoabs.34.P204

P205

Cross-sectional survey of depression and anxiety in a clinical diabetes population: prevalence and role in blood sugar control

Hammad Lakhani[1], Isobel Cane[1], Michelle Taylor[2], Paul Chadwick[2] & Miranda Rosenthal[2]
[1]University College London, London, UK; [2]Royal Free Hospital, London, UK.

Aims
To evaluate the prevalence of depression and anxiety in adult outpatients with diabetes and identify the correlation with diabetes management behaviours and clinical outcomes.
Methods
Cross-sectional analyses were performed on 142 diabetic patients (type 1 diabetes $n = 67$, type 2 diabetes $n = 60$) attending outpatient clinic. Depression and anxiety were evaluated using the Patient Health Questionnaire (PHQ-2) and the General Anxiety Disorder Questionnaire (GAD-2). Validated survey items were used to measure patient characteristics and clinical outcomes.
Results
Prevalence of psychological symptoms was high, with 39 patients (27.3%) having a score indicative of depression as compared to < 10% in the general population, and 29 (20.3%) with a score suggestive of general anxiety disorder. Regression analysis showed that anxiety and quality of life correlated strongly with depression ($R^2 = 0.59$ and 0.43, respectively). At multi-variate analysis, depression and anxiety were significantly associated with higher self-reported blood glucose levels, greater number of diabetes related complications ($P < 0.01$) and less physical activity ($P < 0.05$). Poorer satisfaction with treatment and a lack of self-monitoring of glucose also correlated with depression ($P < 0.05$). More than a half of the patients felt that it was very important for psychological support to be diabetes specific.
Conclusion
In our sample, patients with depression and anxiety had poorer clinical outcomes, with depressed patients also exhibiting worse diabetes management behaviours. This highlights the importance of screening for depression and anxiety in outpatient visits, which would allow for identification and management of depression, thus improving glycaemic control and overall wellbeing.

DOI: 10.1530/endoabs.34.P205

P206

Dark chocolate rich in polyphenols improves insulin sensitivity in the adult non-diabetic population

Grace Farhat
Queen Margaret Univeristy, Musselburgh, East Lothian, UK.

Polyphenols are chemical components found largely in plants. Dark chocolate (DC) is one of the highest sources of polyphenols in foods. Several animal and few human studies have suggested that DC might improve insulin sensitivity and decrease glucose levels. The aim of this study was to determine the effect of polyphenol-rich DC (PRDC) on insulin sensitivity (determined by HOMA and QUICKI) and fasting glucose levels. Sixty-one volunteers with no history of diabetes, hypertension or CVD were enrolled in a randomized controlled parallel trial. Participants randomly received 20 g daily of one of the two different types of DC: placebo DC (with negligible amount of polyphenols) or PRDC (500 mg) for a period of 4 weeks. Anthropometric measurements, blood pressure, blood and saliva samples were collected pre and post intervention. A 3-day diet diary was taken at baseline and at week 3. Data was analysed using a paired Student's *t*-test for within group comparisons and ANCOVA for between group differences (to account for potential baseline imbalances). Results showed a significant lowering effect of PRDC on insulin levels (1.17 ± 1.34 μU/ml, $P < 0.001$), and HOMA-IR (0.2 ± 0.33, $P = 0.003$), and an increasing effect on QUICKI (1.12 ± 0.74, $P < 0.001$), but no significant impact on glucose levels ($P = 0.159$) following the intervention. On the other hand, participants administered placebo showed increases in insulin (0.77 ± 1.56 μU/ml, $P = 0.014$), HOMA-IR (0.27 ± 0.44, $P = 0.003$), and glucose levels (0.44 ± 1.08 mmol/l, $P = 0.041$), with QUICKI levels decreasing (0.35 ± 0.7, $P = 0.013$) after 4 weeks. No changes in blood pressure or salivary glucocorticoid hormones (cortisol and cortisone levels) were noted. These results indicate a beneficial effect of PRDC on improving insulin sensitivity, and possibly on preventing or delaying the onset of diabetes. Results also highlight the potential role of polyphenols in counteracting the negative effects of fat in the diet as previously suggested.

DOI: 10.1530/endoabs.34.P206

P207

Effect of *H. gordonii* on regulatory peptides under short-term calorie restriction

Swati Jain & SomNath Singh
Defence Institute of Physiology and Allied Sciences, Delhi, India.

H. gordonii is a supplement of natural origin indigenous to South Africa and known for its appetite suppressant properties. Owing to its acclaimed anorectic activity, the study was aimed to evaluate the effect of *H. gordonii* supplementation during short-term calorie restriction on appetite regulatory peptides and endocrine markers. Male albino rats were divided into three groups (n = 12 in each) – control, calorie restricted (CR, 25% for 5 days), calorie restricted and *H. gordonii* supplemented (CR + HG, organic solvent extract given orally for 5 days at a dose of 100 mg/kg). The regulatory peptides, i.e. ghrelin, leptin, CCK, NPY, insulin and thyroid hormones were estimated in plasma at the end of the treatment. On comparison with CR rats, changes were noticed in the levels of regulatory peptides of the CR + HG rats. A significant decline in the hunger hormone, ghrelin ($P < 0.05$) and marked increase in leptin levels were observed with a slight inhibitory effect on NPY. This denotes the appetite suppressing activity of the herb. Significantly high concentration of CCK was also noticed in CR + HG group against CR and control ($P <$;0.05). A modest increase in plasma T_3 levels and an unaltered response to the thyroxin levels was observed in the CR + HG rats when compared to the CR. These changes indicate hunger suppression caused by *H. gordonii* under CR.It is concluded that *H. gordonii* can modulate hunger during CR and may be used for better adherence to dietary restriction regime.

DOI: 10.1530/endoabs.34.P207

P208

Abnormal glucocorticoid metabolism in horses with metabolic syndrome

Ruth Morgan[1,2], Patrick Hadoke[1], Brian Walker[1] & John Keen[2]
[1]The Queen's Medical Research Institute, BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK; [2]Royal (Dick) School of Veterinary Studies, University of Edinburgh, Edinburgh, UK.

Activation of the hypothalamic–pituitary–adrenal (HPA) axis and altered tissue glucocorticoid action in obesity and metabolic syndrome has been attributed to altered peripheral cortisol metabolism. In human obesity, cortisol clearance is increased with up-regulation of A-ring reductases and down-regulation of cortisol-regenerating 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in liver, while in adipose tissue 11β-HSD1 is up-regulated. Rodent studies suggest this dysregulation is species-specific. This study addressed whether abnormalities of glucocorticoid metabolism occur in equine metabolic syndrome (EMS).
Morning (0900–1100 h) plasma cortisol and serum insulin concentrations were measured in 11 healthy controls and 12 EMS cases. Adipose (neck crest, perirenal, and linea alba) and liver samples were obtained post-mortem and 11β-HSD1, 11β-HSD2, glucocorticoid (GR) and mineralocorticoid receptor

(MR) mRNA transcript levels quantified by qPCR. Cortisol metabolites were measured by GC–MS in urine aliquots from sub-groups ($n=5$–6) and corrected for creatinine concentration. Data are mean ± S.E.M.
Fasted serum insulin ($P=0.002$) but not plasma cortisol, was increased in EMS. Body condition score (/5) was higher in EMS (control 2.9 ± 0.24 vs EMS 3.8 ± 0.27, $P=0.046$) indicating obesity in this group. 11β-HSD1 mRNA was up-regulated in peri-renal (0.54 ± 0.12 vs 1.25 ± 0.29, $P=0.011$) and linea alba (0.57 ± 0.16 vs 1.55 ± 0.30, $P=0.007$) adipose, but not in neck crest adipose or liver, in EMS. No differences were detected in GR, MR or 11β-HSD2 mRNA levels. Urinary excretion of total cortisol metabolites was increased in EMS ($P=0.041$), with elevated α/β cortols, α/β cortolones, and 20α- and 20β-dihydrocortisol, but lower relative excretion of 5α-tetrahydrocortisol.
In conclusion, obese horses with metabolic syndrome, like humans, have increased cortisol clearance, and increased cortisol regeneration in adipose tissue by 11β-HSD1. The latter may provide a therapeutic target in equine metabolic syndrome. However, dysregulation of cortisol metabolism in equine liver is not mediated by altered 5α-reductase or 11β-HSD1, perhaps reflecting species-specific differences in regulation and/or alternative predominant pathways of glucocorticoid clearance in horses.

DOI: 10.1530/endoabs.34.P208

P209

An audit of patients with diabetes attending accident and emergency with severe hypoglycaemia

Tolulope Shonibare, Khyatisha Seejore & Robert Moisey
Huddersfield Royal Infirmary, West Yorkshire, UK.

Introduction
Hypoglycaemia remains the major barrier to tight glycaemic control in diabetes. Oral hypoglycaemic agents such as sulphonylureas (SU) remain one of the main options for managing type 2 diabetes mellitus (T2DM), but the glucose independent action is associated with an increased risk of hypoglycaemia. We present a retrospective audit of patients attending the A&E Department with severe hypoglycaemia at a large acute trust serving a population of 450 000.
Results
Data was collated using local A&E, electronic patient and GP records. Patients coded as having hypoglycaemia requiring hospital attendance were included. Between August 2008 and February 2012,434 patients attended A&E with a diagnosis of severe hypoglycaemia (229, 53% males). 212 (49%) were admitted and 33 (7.6%) died. The average (range) age was 63.5 (17–99) years, 321 (74%) had T2DM, 113 (26%) had T1DM, one had gestational DM. 109 patients with T2DM (34%) were managed with OHAs alone. Diabetes medication was known in 103. Of these 92 (89.3)% were on a SU (gliclazide; 60.2%, glimepiride; 27.2%, glibencamide; 1.9%). 34 (33%) were taking a SU alone, 51 (49.5%) in combination with metformin. 5 (4.8%) were on metformin alone, 24 (23.3%) were on additional OHAs in combination. 71 (68.9%) of patients on OHAs were admitted to hospital. Compared to those on insulin, patients on OHAs were older 75.9 vs 60.0 years, ($P<0.0001$) with a lower HbA1c of 6.8% vs 8.2%, ($P<0.0001$) and were more likely to be admitted (67 vs 44%). 44 (43%) on a SU had an HbA1c ≤48 mmol/mol and the mean age was 77.6 yearrs.
Discussion
This audit highlights the increased risk of hypoglycaemia associated with SU therapy. Patients on OHAs were older with a lower HbA1c compared to those on insulin. In particular 43% of patients on SU therapy had an HbA1c ≤48 mmol/mol. Although OHA's are considered lower risk for hypoglycaemia, using national targets has resulted in this group of patients being more aggressively managed, putting them at increased risk. We recommend older patients should be given an individualised target for glycaemic control.

DOI: 10.1530/endoabs.34.P209

P210

Predictive clinical variables for periodontitis in type 2 diabetic subjects with poor glycemic control.

Jai Karthik, Shajithanoop Selva kumar, Suresh kumar & M V Usha Rani
Jai Karthik, Coimabatore, Tamilnadu, India.

Type 2 diabetes with poor glycemic control is a predisposing factor for periodontitis (Salvi *et al.* 2000). In this cross sectional study, subjects with type 2 diabetes ($n=232$, mean age: 50.08 ± 3.2 years) were recruited with informed and written consent. Fasting plasma glucose, serum lipid profile and HbA1c was analysed by standardised methods. A complete dental examination for pocket depth, clinical attachment level and periodontitis was performed for all subjects. Clinical data was analysed for statistical significance by Pearson's correlation and linear regression models to determine the predictor variables that determine gingival index in type 2 diabetic subjects with periodontitis ($n=108$) and without periodontitis ($n=123$). In T2DM subjects with periodontitis, the mean HbA1c (7.74%), mean pocket depth (5.79), gingival index (2.86) was comparatively higher than subjects without periodontitis. A positive correlation was observed between gingival index and HbA1C, HDL cholesterol (HDL-C) clinical attachment level ($P=0.001$) and BMI ($P=0.05$). The predictive variables for gingival index were pocket depth ($P=0.000$), HbA1c ($P=0.000$) and total body weight ($P=0.000$) with a regression coefficient (r^2) of 0.67. The observations of the present study infer that HbA1c, HDL, BMI and pocket depth are predictive clinical variables for periodontitis in type 2 diabetes subjects with elevated HbA1c values.

DOI: 10.1530/endoabs.34.P210

P211

Impact of the serum testosterone on the metabolic syndrome in Chinese young and middle-agd men with type 2 diabetes mellitus

Xin Li, Miao Yang, Su-xing Sun, Yu-wen Wu & Jia-zhong Sun
Department of Endocrinology, Zhongnan Hospital, Wuhan University, Wuhan, China.

Objective
To investigate the impact of the serum testosterone on the metabolic syndrome (MS) in young and middle-aged men with type 2 diabetes mellitus (T2DM).
Methods
A total of 220 young-and middle-aged (18–50) men with T2DM were recruited in this study and were divided into four groups based on serum testosterone quartile. General information, smoking history and anthropometry data were collected. Fasting glucose, fasting insulin, blood fat and HbA1c were detected. BMI and homeostasis model assessment of insulin resistance (HOMA-IR) were calculated.
Results
With the increasing of testosterone concentration, the incidence of MS was decreased $X^2=8.748$, $P=0.033$), together with the decrease of age, smoking, course of T2DM, waist circumference, BMI, systolic blood pressure (SBP), diastolic blood pressure (DBP), HbA1c, HOMA-IR, total cholesterol (TC), triglyceride (TG), LDL cholesterol (LDL-C); (all $P<0.05$ or $P<0.01$), and the increase of cholesterol (HDL-C); ($F=1.928$, $P=0.126$). Spearman correlation analyze indicated that serum testosterone was negatively correlated with age ($r=-0.452$), smoking ($r=-0.543$), course of T2DM ($r=-0.314$), waist circumference ($r=-0.417$), BMI ($r=-0.362$), SBP ($r=-0.268$), DBP ($r=-0.275$), HbA1c ($r=-0.329$), HOMA-IR ($r=-0.418$), TG ($r=-0.267$), TC ($r=-0.259$), LDL-C ($r=-0.324$)(all $P<0.05$ or $P<0.01$), and positively correlated with HDL-C ($r=0.078$, $P=0.628$). Logistic regression analyze indicated that serum testosterone was the protective factor of MS in young and middle-aged men with T2DM (OR=0.479, 95%, CI, 0.249–0.936, $P<0.01$) .After the adjustment of age, smoking, course of T2DM, HOMA-IR and HbA1c, the OR was 0.759(95%CI:0.598–0.963 $P<0.05$).
Conclusion
The serum testosterone in young and middle-aged men with T2DM was negatively correlated with MS. Low serum testosterone may be an independent risk factor of MS in such patients.

DOI: 10.1530/endoabs.34.P211

P212

Metabolic improvement after bariatric surgery: an overview from a single tertiary centre

Abigail Barrett, Saloni Bakhshi, Ananth Nayak, George Varughese, Chandra Cheruvu & Lakshminarayanan Varadhan
University Hospitals North Staffordshire NHS Trust, Stoke on Trent, UK.

Aim
Malabsorptive bariatric procedures lead to significant metabolic improvement with ongoing weight loss. The aim of our retrospective analysis was to assess the impact of bariatric surgery on three important metabolic and biochemical abnormalities associated with obesity: diabetes, liver function test (LFT), and dyslipidaemia.
Methods
All patients who had laparoscopic gastric bypass (LGB) or sleeve gastrectomy (SG) over the last 7 years were identified. Patients who did not have sufficient data

or 12 months of follow-up post-operatively were excluded ($n=67$). Data on HbA1c, LFT, and lipids were obtained.
Results
Of the 204 patients identified (169 LGB, and 36 SG) baseline parameters were: age 45 (20–70) years; 28% males; BMI 49.7 (36.9–80.0); excess body weight (EBW) 65.8 (31.5–153.6) kg. The mean duration for follow-up was 526 days. Mean weight loss 45.6(4–104) kg. EBW loss achieved 70.5% (9–145%).
Diabetes
60 patients had diabetes before the surgery (Five diet treated, and 22 insulin treated). 80% of these patients had improvement in diabetes with 45% having complete resolution of diabetes (all with LGB). 19 of the 22 insulin-treated patients came off insulin. Mean number of medication improved from 1.9 (1–5) pre-operative to 0.7 (0–4) at follow-up ($P<0.05$).
Cholesterol
Data available on 146 patients: 25% of the statin-treated patients were weaned off the treatment after surgery. Of the drug naïve patient ($n=126$), 84 had reduction in total cholesterol (mean 1.0 mmol/l, range 0.1–3.9).
LFT
46 patients had abnormal LFT (based on ALT), of whom 80% (n=37) normalised post-surgery.
Conclusion
Bariatric surgery, especially gastric bypass procedures, offers a significant treatment option to achieve clinical remission with various metabolic disorders, which has tremendous implication on morbidity rates and long term costs to healthcare economy.

DOI: 10.1530/endoabs.34.P212

P213

Renal function variation after bariatric surgery: which measurement do we rely on?

Saloni Bakhshi, Abigail Barrett, Ananth Nayak, George Varughese, Chandra Cheruvu & Lakshminarayanan Varadhan
University Hospitals North Staffordshire NHS Trust, Stoke on Trent, UK.

Introduction
Interpretation of renal function in patients with obesity and post-bariatric surgery is a clinical challenge. EGFR could be falsely low and weight loss does not reflect muscle mass. The aim of our study was to compare the fluctuations in renal function assessment in patients undergoing bariatric surgery.
Methods
200 patients who underwent bypass surgery or sleeve gastrectomy were analysed. Six different renal function parameters were analysed using creatinine measured pre-operatively and at-least 12 months post-operatively: EGFR with MDRD-4 variable equation, creatinine clearance (CrCl) with Salazar–Corcoran (SC) equation and CrCl with Cockgroft–Gault (CG) equation using total body weight (TBW), ideal body weight (IBW, adjusted for BMI=25), lean body weight (LBW), adjusted body weight (ABW=LBW+40% of TBW–LBW). CrCl (CG–ABW) has been shown to be the most reliable closest calculated approximation to actual GFR.
Results
Mean age was 46 years and mean follow-up duration was 528 days. Mean excess body weight loss achieved was 70%. Comparison of various parameters before and after surgery (*$P<0.0001$): TBW 135.1 vs 89.6*; BMI 48.8 vs 32.4*; EGFR–MDRD 98.1 vs 104.9 ($P<0.005$); CrCl (SC) 155 vs 132*; CrCl (CG–TBW) 212 vs 148.3*; CrCl (CG–IBW) 108.7 vs 130.0*; CrCl (CG–LBW) 112.5 vs 99.8*; and CrCl (CG–ABW) 145.0 vs 119.2*.
Changes in CrCl with bariatric surgery, measured by (CG–ABW) and CrCl (SC) were comparable to each other; CrCl using other weight variables and eGFR differences between pre and post operative measures, were significantly different in comparison to CrCL (CG–ABW)
Conclusion
Renal function assessment based on CrCl (SC) are comparable to CrCl (CG–ABW). EGFR and CrCl (CG–IBW) underestimates renal function and do not mirror changes expected with GFR after surgery. CrCl (CG–LBW) may underestimate renal function. Routinely used renal function parameters should be interpreted pragmatically in obesity and post-bariatric surgery and has implications with drug-dose adjustments and label of CKD.

DOI: 10.1530/endoabs.34.P213

P214

Chemerin as a potential screening marker for sub-clinical diabetes?

Syeda Sadia Fatima & Najeeha Talat Iqbal
Aga Khan University, Karachi, Pakistan.

Objective
Chemerin a novel adipokine; affects the lipid and glucose homeostasis along with adipose tissue metabolism. Elevated levels of peptide have shown to be associated with disruption of normal insulin function and systemic inflammation, which also results in hyperglycemia in clinical diabetes. This study aims to identify whether chemerin in conjunction with TNFα and hsCRP can act as screening marker to identify subclinical diabetes.
Methods
For this study, 52 asymptomatic healthy volunteers and 22 chronic diabetics (DM) were enrolled. Of the 52 study participants, 23 were classified as as newly diagnosed diabetics on the basis of impaired glucose tolernace test (NDM), subjects with normal glucose tolerance were classified as control ($n=29$).
Results
High chemerin level was observed in 23 NDM ($P<0.01$; ANOVA) compared to controls and DM. A strong positive association was also found between serum chemerin and FBS ($P=0.029$; $r=0.254$). Both the hsCRP and TNF α levels were elevated in subjects with DM compared to controls ($P<0.01$). Similar increase in TNFα levels were also observed in NDM compared to DM ($P<0.001$).
Conclusion
The preliminary findings suggest that chemerin may serve as a potential screening marker in diagnosis of DM or predicting the risk of development of diabetes in asymptomatic individual. Progression to clinical diabetes is associated with an increase inflammatory responses, which usually wanes off in established disease. Further studies with a larger panel of cytokines may predict a biomarker of subclinical diabetes.

Keywords: diabetes, cytokines, inflammation, hsCRP, chemerin
DOI: 10.1530/endoabs.34.P214

P215

The salt-inducible kinases: gatekeeper of hepatic gluconeogenesis

Kashyap Patel[1,3], Marc Foretz[2], David Campbell[1], Calum Sutherland[3] & Kei Sakamoto[1]
[1]MRC Protein Phosphorylation and Ubiquitylation Unit, University of Dundee, Dundee, UK; [2]INSERM, U1016, Institut Cochin, Paris, France; [3]Cardiovascular and Diabetes Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee, UK; [4]Nestlé Institute of Health Sciences SA, Campus EPFL, Quartier de l'innovation, Lausanne, Switzerland.

Regulation of hepatic gluconeogenesis by insulin and glucagon is central to blood glucose homeostasis. It has been proposed that the members of AMP-activated protein kinase (AMPK)-related kinases, the salt inducible kinase (SIK) isoforms, may play a role as signalling mediator in the control of insulin- and glucagon-regulated hepatic gluconeogenesis. However, the exact regulation and contribution of SIKs in hepatic gluconeogenesis is largely elusive. Here we employed selective pan SIK inhibitors (HG-9-91-01 and KIN112) to investigate the role of SIKs in hepatic gluconeogenesis. Both of these inhibitors showed high selectivity against SIKs among other AMPK-related kinases *in vitro*. SIK inhibitor treatment of mouse primary hepatocytes showed robust dose- and time-dependent increase in gluconeogenic gene expression (PEPCK, G6Pase) and glucose production. These effects of SIK inhibitors on gluconeogenesis were validated as SIK-specific using multiple approaches including inhibitor-resistant SIK mutants, LKB1-null hepatocytes (cells where SIK activity was already ablated) and also AMPKα1α2-null hepatocytes. In addition, investigation into hormonal-regulation of SIK2 reveale that SIK2 was phosphorylated on multiple residues in response to glucagon and fasting but not following insulin or refeeding in primary hepatocytes and *in vivo* liver, respectively. Unexpectedly, fasting- or glucagon-stimulated phosphorylation of SIK2 did not modulate SIK2 kinase activity and its subcellular localisation. Thus, these experiments have proposed a novel insight that SIKs function as 'molecular gatekeeper' to suppress hepatic gluconeogenesis in an insulin-independent mechanism and thus contribute significantly towards maintenance of glucose homeostasis.

DOI: 10.1530/endoabs.34.P215

P216

Maturity onset diabetes of young type 5

Ahmed Imran Siddiqi
Colchester Hospital University Foundation Trust, Colchester, UK.

Background
I present a young girl with maturity onset diabetes of young type 5 (MODY 5) who presented with renal cysts and early onset bilateral cataracts with gradual decline of beta cell functions.
Case
19 years old girl presented to A&E with hyperglycemia, ketosis, and acidosis. This was her second presentation in A&E in previous 3 weeks. She was diagnosed with type 1 diabetes when she was 3 years old in view of her polyuria and slow growth. She had normal vaginal delivery without any complications. Her maternal grandmother had type 2 diabetes and no other family member was known to have diabetes. Her glycemic control had always been poor with HbA1c ranging between 10 and 15% (85–140 mmol/mol). She never developed diabetic ketoacidosis prior to these two episodes. She had been treated with twice daily pre-mixed insulin (30% short acting/70% intermediate acting insulin). She was supposed to be taking 34 units in the morning and 38 units in the evening. She had also received metformin in view of her large doses of insulin to improve insulin sensitivity. In past medical history she had her bilateral cataract surgery done at age 14 years. She was found to have urine ACR of 29 mg/mmol on this admission and C-peptide levels were 0.62 ng/ml (0.71–2.72 ng/ml). Her USS of kidneys showed multiple cysts. In view of her atypical presentation for type 1 diabetes and renal cysts she had her genetic analysis done which showed HNF1b mutation (heterozygous) consistent with MODY 5. She did not develop DKA earlier as MODY 5 patients do have some β cell function which gradually decline and hence she started developing DKA now when her β cell function had reduced significantly. Renal cysts are also a well-known feature of MODY5 however none of the previously reported cases in literature have mentioned early onset bilateral cataracts in such patients. Since the MODY5 is rather rare and the clinical manifestations are variable, early onset cataract could be an even less common manifestation of the disease.
Conclusion
Careful history is important in diagnosing patients with MODY and differentiating them from type 1 diabetes. Early onset cataract has not been described in previously reported cases of MODY5.

DOI: 10.1530/endoabs.34.P216

P217

Obstructive sleep apnoea syndrome and type 2 diabetes

Katy Talbot, Peter Evans & Melissa Hack
Cardiff University, Cardiff, Wales, UK.

Introduction
The prevalence of OSAS in the general population is ~ 3%. Associations between T2D and OSAS have been shown, but the prevalence of OSAS in T2D in Wales is not known.
In OSAS recurrent episodes of collapse of the upper airway (apnoeas) occur during sleep. The resulting transient hypoxia and increased sympathetic drive leads to repetitive arousals. Sleep fragmentation results in excessive daytime sleepiness (EDS). Snoring, witnessed apnoeas, and EDS are the main symptoms of OSAS. OSAS is known to be associated with obesity, vascular events and hypertension.
The aim of this study is to assess OSAS in T2D patients.
Method
50 patients attending diabetes clinics at The Royal Gwent Hospital participated in this questionnaire study. The questionnaire incorporated elements from the Berlin Sleep questionnaire, Wisconsin Sleep Questionnaire, and the Epworth Sleep Scale. BMI, HbA1c, and blood pressure data were collected.
Participants were scored for symptoms of OSAS.
Results
Ten patients had symptoms highly suggestive of OSAS. Four participants were already diagnosed and undergoing treatment for OSAS. One participant had three symptoms of OSAS which would indicate referral for investigation.
The prevalence is 11.1%. Five participants had two symptoms of OSAS which would indicate referral for investigation. Including these participants gives a prevalence of 22.2% of participants with significant OSAS symptoms or previously diagnosed OSAS. There was a significant difference with a higher BMI found in the OSAS symptom group ($P=0.04$). There was no statistical difference in Hb1Ac or hypertension between the groups.
Conclusion
The prevalence of OSAS based on this questionnaire study is increased in T2D in Wales compared to the general population. This finding is important as OSAS is a potentially modifiable factor associated with obesity, T2D, and vascular disease.

DOI: 10.1530/endoabs.34.P217

P218

Young women with polycystic ovary syndrome have increased concentrations of circulating annexin V-positive microparticles derived predominantly from platelets

Gareth Willis[1], Katherine Connolly[1], Kristin Ladell[2], Kelly Miners[2], David Price[2], Irina Guschina[3], Aled Clayton[4], Philip James[1] & Aled Rees[1]
[1]Institute of Molecular and Experimental Medicine, Cardiff University, Cardiff, UK; [2]Institute of Infection and Immunity, Cardiff University, Cardiff, UK; [3]School of Biosciences, Cardiff University, Cardiff, UK; [4]Institute of Cancer and Genetics, Cardiff University, Cardiff, UK.

Background
Women with polycystic ovary syndrome (PCOS) may have increased cardiovascular (CV) risk but the mechanisms are unclear. Microparticles (MP), small circulating vesicles released from platelets, monocytes and endothelial cells, are elevated in patients with CV disease, and may increase CV risk through altered cell content and/or increased surface exposure of procoagulant phosphatidylserine (PS).
Aims
To compare MP characteristics between women with PCOS and healthy volunteers (HV) with respect to concentration, size, cell origin, lipid composition, PS and microRNA (miR) content.
Methods
Nanoparticle tracking analysis and flow cytometry (CD41 platelet, CD11b monocyte, CD144 endothelial) were used to determine MP size, concentration, cellular origin and annexin V positivity (PS exposure). Fatty acid analysis was performed by gas chromatography and MP microRNA (miR) expression profiles were compared by microarray.
Results
Compared with HV ($n=18$, age 31 ± 6 years, BMI 30 ± 6 kg/m^2), patients with PCOS ($n=17$, age 31 ± 7 years, BMI 29 ± 6 kg/m^2) had increased MP concentrations (PCOS: 11.45 ± 5 x10^{12}/ml; HV: 9.98 ± 4x10^{12}/ml; $P=0.03$), which correlated with insulin resistance ($r=0.53$, $P=0.03$). MP size was unchanged (PCOS: 123 ± 7 nm; HV: 114 ± 4 nm; $P=0.18$). A greater percentage of MP were annexin V-positive in PCOS patients compared with HV ($83.6\pm18\%$ vs $74\pm24\%$, respectively; $P\leq0.05$), but cellular origin did not differ (CD41+ MP: 99.3+/-0.9% [PCOS]; 98.6+/-2.5% [HV]; $P=0.27$). MP fatty acid concentration (ng per 1x10^6 MP: 0.009 ± 0.009 [PCOS]; 0.012 ± 0.11 [HV]; $P=0.27$) and composition was similar, but 16 of >1,200 low expression miRs were differentially expressed ($P<0.05$), including miRs that target PPAR gamma (miR 1293), hexose-6-phosphate dehydrogenase (miR-551a) and the FSH receptor (miR574-3p).
Conclusions
Women with PCOS have increased circulating concentrations of annexin V-positive MP containing an altered miR cargo which may contribute to increased CV risk.

DOI: 10.1530/endoabs.34.P218

P219

Quality improvement for patients with type 2 diabetes

Finn Edler von Eyben
Center of Tobacco Control Research, Odense, Denmark.

Aims
To evaluate stepped care intervention for patients with type 2 diabetes.
Method
The audit dealt with 191 patients with type 2 diabetes who were treated at a general hospital in Norway between 2007 and 2012. A local database looked after process and results.
Results
The intervention increased the number of antihypertensive drugs, and the dose of metformin and insulin. That lowered mean levels of systolic blood pressure from 142 to 132 mmHg, LDL cholesterol from 2.6 to 2.1 mmol/l, and, HbA1c from 8.5 to 7.6% (64–57 mmol/mol, $P<0.001$, t-tests). In multiple linear regression analyses, decrease of the three metabolic risk factors was significantly associated

with first level of the risk factors and the number of visits or follow-up. One patient had severe hypoglycaemia but else toxicity was moderate. Ten patients died.
Conclusions
Local computerized surveillance of the intervention helped to improve the complex treatment for poorly controlled type 2 diabetes. The improvement continued over four years of follow-up.

DOI: 10.1530/endoabs.34.P219

P220

Central arterial stiffness and diastolic dysfunction are not increased in young women with PCOS but are associated with insulin resistance and abdominal obesity

Rosie Coulson[1], Emma Rees[2], Frank Dunstan[3], Julian Halcox[4], Alan Fraser[1] & Aled Rees[1]
[1]Institute of Molecular and Experimental Medicine, Cardiff University, Cardiff, UK; [2]College of Human and Health Sciences, Swansea University, Swansea, UK; [3]Institute of Primary Care and Public Health, Cardiff University, Cardiff, UK; [4]Institute of Life Sciences, Swansea University, Swansea, UK.

Background
Polycystic ovary syndrome (PCOS) is characterised by a high prevalence of obesity and insulin resistance, leading to an increased risk of type 2 diabetes. However, it is unclear whether cardiovascular risk (CVR) is increased in PCOS independently of these metabolic disturbances.
Aims
To establish whether PCOS is associated with arterial stiffness, carotid intima media thickness (cIMT) and myocardial dysfunction, and explore the relationships of these CVR markers with body composition and insulin sensitivity.
Methods
Subcutaneous and visceral fat (VF) area was measured by CT. Insulin sensitivity was established by insulin response (IAUC) following glucose challenge. Arterial stiffness (aortic pulse wave velocity (aPWV), cIMT and tissue Doppler-derived diastolic (e':a') myocardial velocities were used as surrogate markers of CVR.
Results
84 women with PCOS and 95 healthy volunteers (HV) (age 16–45) were included. After adjustment for age and BMI, PCOS subjects had higher IAUC (adjusted difference (AD) 35950 pmol min/l, $P<0.001$), testosterone (AD 0.57 nmol/l, $P<0.001$) and adiponectin (AD 3.01 μg/ml, $P=0.02$), but no significant differences in aPWV (AD −0.13 m/s, $P=0.33$), cIMT (AD −0.01 mm, $P=0.13$) or e':a' (AD −0.01, $P=0.86$). In regression analyses, after adjustment for age, height and central pulse pressure, e':a' (standardised regression coefficient (SRC) −0.391, $P<0.0001$) and aPWV (SRC 0.293, $P<0.0001$) were associated with logVF but the association between logVF and cIMT was not significant (SRC 0.078, $P=0.28$). e':a' (SRC −0.469, $P<0.0001$) and aPWV (SRC 0.31, $P<0.0001$) were also independently associated with IAUC. The relationships between e':a' or aPWV and logVF were only partly attenuated by adjusting for insulin sensitivity (e':a' SRC −0.173, $P<0.0001$and aPWV SRC 0.214, $P=0.015$). There was no significant relationship between a PWV or e':a' and either testosterone or adiponectin.
Conclusions
Insulin resistance and central obesity are associated with subclinical cardiovascular dysfunction in young women. A diagnosis of PCOS does not confer additional risk.

DOI: 10.1530/endoabs.34.P220

P221

An evaluation of knowledge and understanding of HbA1c in diabetes patients in a secondary care setting

Caroline Bowler[1], Ruth Erde[1] & Parijat De[2]
[1]University of Birmingham, Birmingham, UK; [2]Department of Diabetes and Endocrinology, City Hospital, Birmingham, UK.

Aim
NICE guidelines states that every diabetes patient should have sufficient knowledge of HbA1c to allow them to be actively involved in their diabetes management. Our study aimed to investigate this in an outpatient setting in a multi-ethnic UK hospital clinic.
Method
We interviewed 97 patients using an out-patient questionnaire and also gathered demographic information and latest HbA1c values from electronic data and medical records.
Results
Only 38% (95% CI=28.6–48.6) of our study population sample ($n=97$) reported familiarity with HbA1c (47 females and 29% males, $P=0.072$).Those with type 1 diabetes were more likely than type 2 to recognise the term HbA1c (77% compared to 32%, $P=0.002$). Those with higher educational attainment ($P=0.002$), and from more skilled occupations (NS-SEC), $P=0.004$, were significantly more likely to have heard of HbA1c. Patients self-reporting Asian ethnicity were the least likely to have heard of HbA1c (19% Asians compared to 49% Caucasian, 42% Afro-Caribbean and, 67% 'Others' $P=0.037$).
There was no significant relationship between the patients' most recent HbA1c value across a range of recorded variables: age ($P=0.0114$), gender ($P=0.159$), diabetes type($P=0.725$), ethnicity ($P=0.292$), employment classification ($P=0.564$), time since diagnosis ($P=0.201$), and education level ($P=0.232$). HbA1c value did not differ significantly according to knowledge of HbA1c ($P=0.092$) either.
Conclusion
Basic knowledge of HbA1c amongst our clinic population is significantly limited. Unlike other studies (USA and Scandinavia), our results do not show that greater patient knowledge of HbA1c necessarily translates into improved glycaemic control. Better understanding of their own HbA1c and thereby long-term diabetes control, is fundamental to self empowerment of diabetes patients. We need to emphasise more on practical aspects of patient education including use of materials and resources in a variety of languages if we are to alter long term outcomes in our diabetes population.

DOI: 10.1530/endoabs.34.P221

P222

Joint predictability of physical activity and bodyweight on health-related quality of life among Nigerian type 2 diabetes

Kolade Idowu
Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria.

Purpose
The study was undertaken to investigate the impact of physical activity (PA) and body weight on health-related quality of life (HRQoL) among type 2 diabetes mellitus attending a tertiary health facility in Nigeria.
Relevance
Despite numerous health benefits of PA, its promotion is often inadequate. Majority of this population do not become or remain regularly active. This study provides additional evidence of importance of regular PA.
Participants
Consecutive sample of 119 participants with mean age (61.8±11.8 years) were selected. They included 47 men (39.5%) and 72 women (60.5%).
Methods
A cross-section of the participants' PA were assessed using long form of International Physical Activity Questionnaire and were categorized as physically active or inactive. Their quality of life was assessed with health survey short form-36 (SF-36) questionnaire.
Results
About 62% of the participants were overweight or obese while 69% were physically active and 31% inactive. There was sex difference in BMI but not in physical activity. Role limitation physical and role limitation emotional were most affected domains of HRQoL. The HRQoL of physically active were significantly higher than of physically inactive participants in all domains except pain domain. The HRQoL of obese participants were lower than overweight participants but only significant for pain domain. Physical activity (met min/week) was significantly correlated with all domains of HRQoL with exception of emotional wellbeing and pain domains. Regression analysis revealed that physical activity remains a significant predictor of physical composite summary ($R^2=0.16$; $P<0.001$), physical functioning ($R^2=0.21$; $P<0.001$), role limitation physical ($R^2=0.14$; $P<0.001$), general health ($R^2=0.13$; $P<0.01$), mental composite summary ($R^2=0.13$; $P<0.01$), role limitation emotional ($R^2=0.11$; $P<0.01$) and emotional well-being ($R^2=0.09$; $P<0.05$) when controlling for age, sex, and BMI.
Conclusions
High level of PA improves HRQoL while increased BMI decreases it. Future studies should compare normal population with T2DM.

DOI: 10.1530/endoabs.34.P222

P223

A 3-month low fat diet leads to significant lipid profile improvement in obese T2DM Saudi subjects, without substantial weight loss, and the capacity to manage a damaging high-fat meal challenge more appropriately post intervention

Dara Al-Disi[1,2], Nasser Al-Daghri[1], Nasiruddin Khan[1], Mohammad Alsaif[1], Assim Alfadda[1], Shaun Sabico[1,2], Gyanendra Tripathi[1] & Philip McTernan[1]
[1]King Saud University, Riyadh, Saudi Arabia; [2]Warwick Medical School, Coventry, UK.

Background and aims
Current evidence highlights that dietary cholesterol, trans-fatty acids and saturated fatty acids (SFAs) are all known to increase the levels of systemic atherogenic lipoproteins and cardiovascular disease. The aim of this study was to observe the direct effect of dietary change, via a calorie-restricted diet on i) cardio-metabolic profile and ii) a high-fat meal challenge pre- and post-3-month intervention.
Methods
T2DM subjects (Saudi female, age: 40.50±6.8years, BMI: 37.28±10.75 kg/m^2, $n=18$) were given a high-fat meal pre- and post-calorie restricted diet (3 months; 500 kcal deficit/day, balanced diet with complex carbohydrate). Baseline (0 h) and post-prandial sera (1–4 h) were taken from subjects, anthropometric and biochemical data was collated at both time points.
Results
On baseline comparison of pre- and post-diet interventions, there were modest reductions in anthropometric data, BMI ($P<0.001$), waist ($P<0.001$), and waist:hip ratio (WHR; $P<0.01$). Baseline HDL-cholesterol increased significantly ($P<0.01$) whilst LDL- and total-cholesterol were significantly reduced (pre-total cholesterol: 5.13 (4.53, 5.93) vs post-total cholesterol: 4.70 (4.01, 5.14); pre-LDL cholesterol: 3.56 (3.07, 4.06) vs post-LDL cholesterol: 2.81 (2.34, 3.56), $P<0.05$). The findings also showed significant changes in the effects of high-fat meal intake on the metabolic profile pre- and post-diet intervention. At 4 h post-prandially, post-dietary intervention, HDL-cholesterol was 16.6% higher than pre-diet ($P<0.05$), whilst LDL- and total-cholesterol were 24.2 and 12% lower, respectively, than at the 4 h equivalent pre-diet ($P<0.05$).
Conclusions
These findings suggest that lipid mediators associated with increased cardiometabolic risk can be quickly reversed as a result of a balanced diet, in T2DM subjects without substantial weight loss. As a result, the body is able to cope with the occasional high-fat meal insult, whilst still maintaining a reduced long-term CVD risk. As such, this is a diet that patients with T2DM may be able to adhere to more successfully, longer-term.

DOI: 10.1530/endoabs.34.P223

P224

DLK1/PREF1 prevents hepatosteatosis by elevating pituitary GH secretion

Marika Charalambous[1,2], Simao daRocha[1,3] & Anne Ferguson-Smith[1]
[1]University of Cambridge, Cambridge, UK; [2]Queen Mary University of London, London, UK; [3]Institute Curie, Paris, France.

GH deficiency is clinically associated with non-alcoholic fatty liver disease. Here we describe a new *in vivo* model of pituitary GH modulation resulting in protection from hepatosteatosis, by over-expression of delta-like homologue 1 (*Dlk1/Pref1*) from endogenous control elements. This resulted in improved glucose tolerance with no primary defect in adipose tissue expansion, even under extreme metabolic stress. Rather, *Dlk1* overexpression caused pituitary IGF1 resistance and hence a defect in feedback regulation of GH. Circulating GH was elevated and accompanied by several physiological sequelae culminating in a switch in whole body fuel metabolism to favour fatty acid utilisation. Elevated serum DLK1 is observed in physiological states of high FA utilisation such as during pregnancy and before weaning. We propose that the normal function of DLK1 is to shift the metabolic mode of the organism toward peripheral FA oxidation and away from lipid storage, thus mediating important physiological adaptations associated with early life.

DOI: 10.1530/endoabs.34.P224

P225

Outcomes of bariatric surgery in men versus women: a matched observational cohort analysis

Andrew Kennedy-Dalby[1,2], Basil Ammori[1,2] & Akheel Syed[1,2]
[1]Faculty of Health and Medical Sciences, University of Manchester, Manchester, UK; [2]Salford Royal NHS Foundation Trust, Manchester, UK.

Background
The global uptake of bariatric surgery in men, despite high prevalence of morbid obesity and associated co-morbidities, is significantly lower compared to women. It is unclear if this is due to a perception of poorer outcomes in men.
Aims
To compare weight loss and metabolic outcomes in men versus women after bariatric surgery.
Design and methods
We performed a retrospective, observational cohort analysis of 80 men matched to 80 women for baseline age (± 5 years), body mass index (BMI) (± 2 kg/m^2), type of bariatric procedure, presence of type 2 diabetes, and continuous positive airway pressure (CPAP) therapy for obstructive sleep apnoea.
Results
Baseline mean ± standard deviation age in men versus women was 46.4±9.9 vs 46.1±10.2 years, and BMI 52.1±7.4 vs 52.3±7.5 kg/m^2. The BMI reduced to 33.± 6.4 vs 29.7±5.8 kg/m^2 at 36 months post-bariatric surgery (ns, non-significant) (Figure 1). Type 2 diabetes was present in 34 men matched to 34 women; HbA1c was 71.0±19.8 vs 61.0±19.8 mmol/mol at baseline (ns) and reduced to 35.0±6.2 vs 46.0±15.5 mmol/mol at 24 months (ns). Reductions in systolic and diastolic blood pressures, total cholesterol:HDL cholesterol ratio and CPAP usage were also similar in men vs women.
Conclusions
Weight loss and metabolic outcomes after bariatric surgery are of similar magnitude in men compared to women. The uptake of bariatric surgery in eligible men should be encouraged.

DOI: 10.1530/endoabs.34.P225

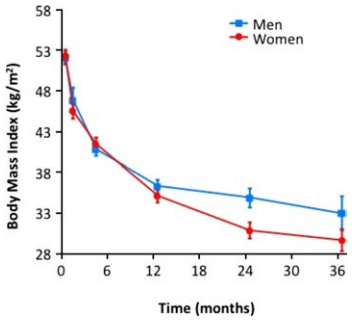


Figure 1 Reduction in body mass index after bariatric surgery in men versus women. Data points ± error bars represent mean ± standard error.

P226

Meal size and frequency influences metabolic endotoxaemia and inflammatory risk but has no effect on diet induced thermogenesis in either lean or obese subjects

Milan Piya[1,2], Narendra Reddy[1,2], Alison Campbell[2], John Hattersley[2], Louise Halder[2], Gyanendra Tripathi[1], Abd Tahrani[3], Thomas Barber[1,2], Sudhesh Kumar[1,2*] & Philip McTernan[1]
[1]Division of Metabolic and Vascular Health, Warwick Medical School, University of Warwick, Coventry, UK; [2]Human Metabolism Research Unit, University Hospitals Coventry and Warwickshire NHS Trust, Coventry, UK; [3]Centre for Diabetes, Endocrinology and Metabolism, University of Birmingham, Birmingham, UK.

Background
Small frequent meals are often recommended for weight loss, with supporting evidence often provided from studies in diabetes. Dietary meal content is also relevant, as high fat meals cause systemic inflammation via gut derived bacteria, endotoxin. As such, repeated meals may exacerbate this. In contrast, dietary induced thermogenesis, related to meal size, may reduce with small frequent meals.

Aim
Therefore, the aim of this study was to compare the effect of 2 vs 5 meals on metabolic endotoxaemia and 24 h (hour) energy expenditure in lean and obese women.
Methods
In a crossover study, 24 lean (age: 34 (mean ± s.d.) ± 10 years, BMI: 22.9 ± 2 kg/m^2) and obese (age: 42 ± 9 years, BMI: 36 ± 8 kg/m^2) women were given two or five isocaloric high (50%) fat meals, on two separate days. On both visits, 24 h energy expenditure was measured in whole body room calorimeters and blood samples taken 2 hourly (0900 to 2100 h). Serum endotoxin, glucose, insulin, lipids were measured.
Results
The obese subjects had increased area under the curve (AUC) for insulin, glucose, HOMA-IR and triglyceride (TG), with decreased HDL ($P<0.01$), compared with lean subjects, for both meal visits. For the entire cohort, fasting endotoxin correlated with triglyceride ($r=0.32$, $P<0.05$), and AUC for endotoxin and TG correlated in the five meal visit ($r=0.44$, $P<0.05$), but not the two meal visit. In the final 2100 h blood test, the endotoxin levels were significantly higher in the five meal visit ($P=0.05$), but not the two meal visit. Meal frequency did not affect 24 h expenditure, in either the obese group (2124 ± 312 vs 2142 ± 365 Kcal/day) or lean group (1724 ± 160 vs 1683 ± 166 Kcal/day).
Conclusion
Our findings suggest in metabolically healthy lean and obese subjects, increased meal frequency may pose an inflammatory risk posed by circulating endotoxin and TGs leading to peak levels at bedtime. As such, small frequent meals may not influence diet induced thermogenesis, but may increase metabolic disease risk.

DOI: 10.1530/endoabs.34.P226

P227

Screening and diagnosis of gestational diabetes: impact of age, BMI and adipokines

Rym-Ikram Mehaoudi[1], Hanane Oussekhri[1], Yacine Soltani[1], Djouhra Tamdrari[2], Hafida Bendaoud[3], Zohra Hamouli[1], Zohra Niboucha[1] & Fatima Hadj Bekkouche[1]
[1]Laboratory of Biology and Physiology or Organisms, Faculty of Biological Sciences, USTHB, Algiers, Algeria; [2]Mother-Child Clinic- Smar, Algiers, Algeria; [3]High-Risk Pregnancies Service- Department of Gynecology, University Hospital Centre Nafissa Hamoud, HusseinDey, Algiers, Algeria.

Background
The onset of gestational diabetes (GD) is closely related to hormonal and metabolic parameters and environmental predisposition.
Aims
This first study of screening and diagnosis of GD in the Algerian population compares the changes in metabolic and endocrine parameters in relation to the state of insulin resistance, BMI and age between women diagnosed positive (GD) and negative (control).
Patients and methods
After HbA1c test, O'Sullivan oral glucose tolerance test (O'Sullivan OGTT 100 g) was performed on 36 women aged 20–45 years, at 24–28 weeks amenorrhea admitted in the high-risk pregnancies service. Fasting blood glucose, lipidemia, insulin, estradiol, progesterone, cortisol, human placental lactogen (HPL), leptin, adiponectin, and HOMA-IR were compared in both groups.
Results
In absence of previous type 2 diabetes (HbA1c <6), screening reveals four GD patients with insulin resistance (HOMA-IR: 7.48 ± 2.435 vs 4.44 ± 0.438), a slight rise in lipidemia, an increase of both leptin and adiponectin by 33 and 88% respectively and a higher adiponectin/leptin ratio in GD group (0.67 vs 0.56). Oestradiol, progesterone, HPL, cortisol levels show a slight increase BMI reveals no obese status among GD group (thin and overweight), against 15.6% obese and 34.3% overweight in control group, while GD two were aged under 35 years vs 75%. Diabetic profile disappeared after delivery.
Conclusions
The risk of gestational diabetes depends upon BMI and age. The increased level of adiponectin and adiponectin/leptin rate consequently to the absence of obesity among GD women seems to mitigate the severity of insulin resistance and may play a protective role against the achievement of a *postpartum* type 2 diabetes.

DOI: 10.1530/endoabs.34.P227

P228

Abstract Withdrawn.

DOI: 10.1530/endoabs.34.P228

P229

The effects of treatment with Liraglutide on atherothrombotic risk in obese young women with polycystic ovary syndrome and controls

Hassan Kahal[1], Ahmed Aburima[1], Tamas Ungvari[2], Alan Rigby[1], Anne-Marie Coady[2], Rebecca Vince[3], Ramzi Ajjan[4], Eric Kilpatrick[2], Khalid Naseem[1] & Stephen Atkin[1]
[1]Hull York Medical School, Hull, UK; [2]Hull and East Yorkshire Hospitals NHS Trust, Hull, UK; [3]University of Hull, Hull, UK; [4]Leeds Institute for Genetics, Health and Therapeutics, University of Leeds, Leeds, UK.

Introduction
Polycystic ovary syndrome (PCOS) is associated with obesity and increased cardiovascular (CV) risk markers.
Objective
To assess the effects of 6 months treatment with liraglutide 1.8 mg od on obesity, and CV risk markers, particularly platelet function, in young obese women with PCOS compared to matched controls.
Design
Interventional case–control study. Carotid intima-media wall thickness (cIMT) was measured by B-mode ultrasonography, platelet function by flow cytometry, clot structure/lysis by turbidimetric assays and endothelial function by ELISA and post-ischaemic reactive hyperemia (RHI).
Results
Nineteen obese women with PCOS and 17 controls, age and weight matched, were recruited; baseline atherothrombotic risk markers did not differ between the two groups. Twenty-five (69.4%) participants completed the study (13 PCOS, and 12 controls). At 6 months, weight was reduced by 3.0 ± 4.2 ($P<0.01$) and 3.8 ± 3.4 kg ($P<0.01$) in the PCOS and control groups, respectively. Similarly, HOMA-IR, triglyceride, hsCRP, urinary isoprostanes, serum endothelial adhesion markers (sP-selectin, sICAM and sVCAM), and clot lysis area were significantly reduced in both groups compared to baseline. Basal platelet P-selectin expression was significantly reduced in the control group 0.42 ± 0.2 vs 0.24 ± 0.2, $P=0.02$, while no significant change was noted in the PCOS group 0.52 ± 0.3 vs 0.40 ± 0.3, $P=0.12$. No significant changes were noted in cIMT or RHI in either group.
Conclusions
Liraglutide was associated with 3–4% weight loss in young obese women, with and without PCOS; that reflected in a significant reduction in atherothrombosis markers including inflammation, endothelial function and clotting. Platelet activation was only reduced in the control group, suggesting that platelets from women with PCOS might be more resistant to the effects of liraglutide, and/or associated weight loss.

DOI: 10.1530/endoabs.34.P229

P230

MIneral nutrients and metabolic health: data on nigerian local dishes

Ayodele Morakinyo, TItilola Samuel & Olufeyi Adegoke
College of Medicine of the University of Lagos, Lagos, Nigeria.

Virtually all biochemical processes in the body require dietary micro-nutrients and imbalance in any of these essential of the diet have the potential to impair metabolic activities. Deficiencies of several mineral nutrients result in stunting, down-regulated immune responsiveness and altered metabolic activities. Appropriate dietary intake of these mineral nutrients can address this deficiency and is currently recommended as part of a lifestyle intervention for the prevention of many metabolism-related health problems including diabetes, obesity, high cholesterol and insulin resistance. Given the lack of adequate data on the mineral adequacy of our local dishes for optimal health, the present study was designed

to analyse the mineral composition of local dishes that are widely consumed in Nigeria. A list of local foods commonly consumed in Nigeria was generated; composite samples prepared from primary sample collected were analysed for dietary minerals using harmonized standard procedures. A set of new data on the Na, K, Ca, P, Mg, Mn, Cl, Fe, Cl and Zn contents of 25 local dishes were generated for the first time in this study. Overall, there were large variations in the mineral contents of the dishes analysed, ranging from 6.3 mg/kg (Jollof rice) to 22.2 mg/kg (Yam pottage with beans) for Fe; 5.0 mg/kg (Yam with omelette) to 21.6 mg/kg (Eba with Okro soup) for Na; 7.30 mg/kg (Pounded yam with Egusi) to 19.8 mg/kg (Vegetable soup) for Ca; 4.6 mg/kg (Wanke) to 11.2 mg/kg (Eba with Okazi soup) for K; 2.2 mg/kg (Yam with omelette) to 6.99 mg/kg (Eba with Okazi soup) for Mn; and 74.5 mg/kg (Amala lafun) to 2301 mg (Edikiakong) for Cl. These data will serve as an important resource in future national and international food composition surveys, inform clinicians, health professionals and dieticians to target provision of dietary advice towards the prevention of non-communicable metabolic diseases due to critical mineral imbalances.

DOI: 10.1530/endoabs.34.P230

P231

EZH2 regulates responses of endothelial cells under hypoxia and during tissue regeneration following limb ischemia

Tijana Mitić[1], Micol Marchetti[1], Marco Meloni[1], Andrea Caporali[2] & Costanza Emanueli[1]
[1]Bristol Heart Institute, University of Bristol, Bristol, UK; [2]Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK.

Objectives
Endothelial cells (ECs) have major role in post-ischemic angiogenesis. EZH2 (enhancer of zest homology) carries out trimethylation of lysine 27 on histone3 ($H3K27me^3$) – repressive mark, thus modulating gene expression. We tested if the EZH2 inhibitor 3-deazaneplanocin (DZNep) regulates angiogenesis under hypoxia and limb ischemia (LI) *in vivo* in ECs and mouse limb muscles respectively.
Methods
Human umbilical vein ECs (HUVECs) were cultured in hypoxia (1.2% O_2, 6–48 h), to mimic ischemia, and treated with DZNep (ctr: 1% DMSO) or transfected with siEZH2 (ctr: scramble oligos). Migration was assessed by scratch assay, and network formation by Matrigel. Levels of eNOS and BDNF were measured by qPCR and western blot and ELISA respectively. Chromatin-immunoprecipitation of EZH2 and $H3K27me^3$ at eNOS and BDNF promoters was coupled with qPCR. Further, limb ischemia was used as a mouse model of *in vivo* angiogenesis. CD1 male mice (12 weeks) received DZNep (1.5 mg/kg per 2 days) or vehicle 1 day pre-LI. Post-ischemic blood flow (BF) recovery was assessed by colour laser Doppler. Muscle tissue was collected for immuno-histochemistry and for sorting endothelial like $CD146^+$ cells to measure mRNA expression. We also tested whether DZNep influences mobilisation of bone marrow progenitor cells (based on target protein expression). Blood and bone marrow samples were collected for FACS analyses.
Results and conclusions
Hypoxia and ischemia increased levels of EZH2 and $H3K27me^3$ and their enrichment at eNOS and BDNF promoter, while reducing eNOS expression. DZNep/siEZH2 treatment has reversed these effects by increasing the levels of eNOS and BDNF. In addition, inhibition of EZH2 improved HUVEC migration and network formation. Post-ischemic BF recovery (weeks 1–3) has significantly increased, due to DZNep, and increased the capillary density in the LI muscles. DZNep promotes angiogenesis by enhancing eNOS and BDNF expression in endothelial-like cells $CD146^+$ of ischemic muscles. This is mediated through activation of pro-angiogenic hematopoietic cell in the bone marrow like $Sca\text{-}1^+CD11b^+$ and Lin- $c\text{-}kit^+$.

DOI: 10.1530/endoabs.34.P231

P232

High incidence of cardiac involvement in patients diagnosed with phaeochromocytoma: a clinical study using cardiovascular magnetic resonance imaging

Vanessa Ferreira[1], Mafalda Rodrigues[2], Stefan Piechnik[1], Claudia Marini[1], Theodoros Karamitsos[1], Jane Francis[1], Ranjit Arnold[1], Radu Mihai[3], Julia Thomas[4], Maria Herincs[4], Marta Korbonits[4], Zaki Hassan-Smith[5], Wiebke Arlt[5], Niki Karavitaki[2], Ashley Grossman[2], Stefan Neubauer[1] & John Wass[2]
[1]Division of Cardiovascular Medicine, Radcliffe Department of Medicine, John Radcliffe Hospital, University of Oxford, Oxford, UK; [2]Department of Endocrinology, Radcliffe Department of Medicine, Oxford Centre for Diabetes, Endocrinology and Metabolism (OCDEM), Churchill Hospital, University of Oxford, Oxford, UK; [3]Department of Surgery, Oxford University Hospitals NHS Trust (OUH), John Radcliffe Hospital, Oxford, UK; [4]Department of Endocrinology, Barts and the London School of Medicine, Queen Mary University of London, London, UK; [5]School of Clinical and Experimental Medicine, Centre for Endocrinology, Diabetes and Metabolism (CEDAM), University of Birmingham, Birmingham, UK.

Background
In patients with phaeochromocytoma, sudden and/or chronic exposure to catecholamines may predispose to cardiac pathology, including left ventricular (LV) hypertrophy, myocardial infarction, stress-induced cardiomyopathy and heart failure. We conducted the first prospective, multicentre study using cardiovascular magnetic resonance (CMR) imaging to describe the variety and incidence of cardiac abnormalities in phaeochromocytoma.
Methods
Fifty patients diagnosed with phaeochromocytoma were included. We prospectively recruited patients newly-diagnosed with phaeochromocytoma ($n=20$) with CMR before and after surgery (median 1 year follow-up). Previously-diagnosed patients who had curative surgery ($n=30$) were also recruited. Patients with known cardiac conditions were excluded. CMR included cine imaging for LV function, T2-weighted imaging for oedema and late gadolinium enhancement imaging to detect scarring.
Results
In newly-diagnosed patients, the mean LV ejection fraction was $67\pm10\%$ (range 47–88%; normal 57–81%); 20% ($n=4/20$) had mild global LV dysfunction (EF=47–56%). A significant proportion (65%, $n=13/20$) demonstrated scarring, all with a non-ischaemic pattern (midwall/subepicardial/patchy), but the areas were small (<10% myocardium); no patient had myocardial infarction (subendocardial scarring). One patient demonstrated global myocardial oedema with normal EF. All LV dysfunction or oedema normalised in postoperative follow-up. Previously-diagnosed patients had a slightly higher EF of $73\pm7\%$ (56–86%) compared to newly-diagnosed patients ($P<0.03$); only one (3%) had mild global LV dysfunction (EF=56%). A significantly smaller proportion of previously-diagnosed patients (17%, $n=5/30$; $P<0.001$) demonstrated areas of scarring, which again were small with a non-ischaemic pattern, except for one patient who suffered a small myocardial infarction.
Conclusion
Cardiac involvement is common (65%) in patients newly-diagnosed with phaeochromocytoma, including small areas of non-ischaemic scarring, mild LV dysfunction and myocardial oedema – the latter two demonstrating full reversibility and normalization post surgical resection of the phaeochromocytoma. In patients previously undergone curative surgical resection, the incidence of cardiac abnormalities is lower (17%), predominantly consisting of small areas of non-ischaemic fibrosis.

DOI: 10.1530/endoabs.34.P232

P233

Microparticle profile in patients with type 2 diabetes

Judit Konya[1], Leigh A Madden[2], Thozhukat Sathyapalan[1], Eric S Kilpatrick[3] & Stephen L Atkin[1]
[1]Department of Diabetes Research, University of Hull, Hull, UK; [2]Postgraduate Medical Institute, Centre for Biomedical Research, University of Hull, Hull, UK; [3]Department of Clinical Biochemistry, Hull and East Yorkshire Hospitals NHS Trust, Hull, UK.

Introduction
Microparticles (MPs) are membrane sheds formed during cell activation or apoptosis and are characteristic to the cell of origin. Microparticles are thought to have procoagulant activity and a potential role in the inflammatory process. They have also been identified as potential cardiovascular risk factor markers. Microparticles are found to be elevated in patients with type 2 diabetes mellitus (T2DM). Annexin-V is a general apoptosis marker that binds to phosphatidylserine that is expressed on the surface of apoptotic/activated cells and MPs. Endothelial dysfunction is one of the most important features in type 2 diabetes that contributes to the increased cardiovascular risk in this group of patients.
Aim
To compare the MP profile of patients with T2DM and healthy volunteers. Analysis was performed by flow cytometry.
Results
Nine patients with type 2 diabetes (7M, 2F, aged 64.44 ± 0.51, diabetes duration 5.17 ± 43.44 years) treated by diet ($n=1$) or metformin ($n=8$) and nine healthy

volunteers (7F, 2M, aged 64.44±11.68) participated in the study. MPs were characterised by general cell surface markers and defined as positive events. Platelet (CD42b), leukocyte (CD45), and endothelial (CD106 and CD144) MPs showed a trend to be higher in patients with T2DM however only CD106 reached statistical significance (healthy volunteers, T2DM: platelet derived CD42b MPs: 4705±5363 vs 5302±3719/μl, $P=0.79$; leukocyte CD45 MPs: 180±178 vs 230±154/μl, $P=0.54$; endothelial CD106 MPs: 174±115 vs 415±197/μl, $P<0.01$; endothelial CD144 MPs: 149±125 vs 630±845/μl; P 0.11, overall MPs: 5203±5340 vs 6577±3892/μl, $P=0.54$).

Conclusion

Patients with type 2 diabetes have elevated number of CD 106 MPs that are recognised as an emerging marker of endothelial cell dysfunction.

DOI: 10.1530/endoabs.34.P233

P234

Expression of GLP1 receptors throughout the mouse brain using a novel transgenic mouse model

Simon C Cork[1], Frank Reimann[2], Fiona M Gribble[2] & Stefan Trapp[1]
[1]UCL, London, UK; [2]Cambridge Institute for Medical Research, Cambridge, UK.

Glucagon-like peptide 1 (GLP1) acts as both a peripheral incretin hormone and a central neuropeptide to regulate glucose and energy homeostasis. Within the brain, GLP1 is synthesised by a discrete collection of neurones in the brainstem, and presynaptic release of GLP1 results in binding to postsynaptic GLP1 receptors (GLP1R). The pattern of projections from these GLP1 synthesising neurones in the mouse brain has been described previously[1,2]. Here we use a novel transgenic mouse expressing Cre under the control of the GLP1R promotor with an ROSA26-tdRFP reporter to investigate brain regions which express the receptor for GLP1. GLP1R-Cre mice were transcardially perfused with 0.1 M PB followed by 4% paraformaldehyde. Brains were removed and sectioned to 30 μm before immunofluorescent detection of tdRFP. RFP-positive cells were found throughout the rostrocaudal extent of the brain in areas which correlate with those previously reported in rat[3]. Specifically, high levels of RFP-positive cells were found in the dorsomedial hypothalamus, paraventricular nucleus of the hypothalamus, ventral tegmental area, and area postrema. This also correlates with areas which receive high levels of input from GLP1 neurones[2]. Interestingly, RFP positive neurones were also found in areas devoid of PPG-neurone projections, such as the hippocampus and cortex, raising the question whether these areas may respond to GLP1 of non-neuronal origin. The results from this study provide further information regarding the distribution of GLP1Rs in the mammalian brain. Furthermore, the use of Cre in neurones expressing GLP1R provides a molecular handle on these neurones in future investigations.

References

1. Llewellyn-Smith *et al. Neuroscience* 2011 **180** 111–121.
2. Llewellyn-Smith *et al*. *Neuroscience* 2013 **229** 130–143.
3. Merchenthaler *et al*. *J Comp Neurol* 1999 **403** 261–280.

DOI: 10.1530/endoabs.34.P234

P235

Novel syndromes of hypoinsulinaemic, hypoketotic hypoglycaemia

Sarah Leiter[1], Marina Minic[1], Victoria Parker[1], Julie Harris[1], Julian Hamilton-Shield[2], Rachel Williams[3], Eckhard Korsch[4], Khalid Hussain[5] & Robert Semple[1]
[1]Institute of Metabolic Science, University of Cambridge, Cambridge, UK; [2]Royal Bristol Hospital for Children, Bristol, UK; [3]Department of Paediatrics, Addenbrookes Hospital, Cambridge, UK; [4]Department of Paediatrics, Cologne Hospitals, Cologne, Germany; [5]Institute of Child Health, University College London, London, UK.

Congenital hyperinsulinaemic hypoglycaemia is generally characterised by low levels of ketone bodies, fatty acids and branched chain amino acids at the time of severe hypoglycaemia, and by a requirement for a relatively high rate of glucose infusion (>10 mg/kg per min) to maintain euglycaemia. It is caused by physiologically inappropriate insulin secretion from the pancreatic beta cells due to mutations that uncouple insulin secretion from normal hyperglycaemic and other stimuli.

We have recently described a novel form of sustained hypoglycaemia presenting in infancy, featuring a similar metabolic profile to hyperinsulinism, but with undetectable insulin, a lower requirement for glucose infusion and left sided hemihypertrophy. This is due to partial activation of a key arm of the insulin signalling pathway caused by the p.Glu17Lys mutation in the signal-transducing serine/threonine kinase AKT2.

We now present a further case series of five patients presenting in infancy or childhood with hypoglycaemia and a similar metabolic profile in the absence of detectible serum insulin. This severe metabolic derangement was associated with a heterogeneous range of associated clinical features including left sided hemihypertrophy with congenital adrenal carcinoma, symmetrical and severe obesity, small bowel atresia, and facial dysmorphism. Genomic sequencing of lymphocyte DNA, and DA from skin fibroblasts where available, failed to identify any mutations in *AKT2*. Moreover studies of primary dermal fibroblasts in two patients have failed to demonstrate basal hyperphosphorylation of AKT and its substrates GSK3 and PRAS40, and show no evidence of the blunted peak phosphorylation of the same proteins in response to insulin that is seen in primary cells harbouring the *AKT2* mutation.

Collectively these clinical and biochemical studies suggest the existence of a heterogenous group of disorders which are metabolic phenocopies of basal AKT2-dependent hyperactivation of insulin signalling, but without cell autonomous evidence of basal AKT signalling. Further investigation of this group promises to yield novel information.

DOI: 10.1530/endoabs.34.P235

P236

The characteristics of a patient population with extreme and complex obesity attending a specialist weight management service

Wen Bun Leong[1], Peymane Adab[1], Neil G Thomas[1] & Shahrad Taheri[2]
[1]University of Birmingham, Birmingham, UK; [2]Weill Cornell Medical College, Doah, Qatar.

It is important for a specialist weight management service to provide a holistic patient-oriented assessment and treatment plan. To achieve this goal, we performed a service evaluation of the characteristics of patients with extreme and complex obesity attending a specialist weight management service. Anonymised data on 126 patients who attended the Heart of England NHS Foundation Trust specialist weight management service were collected. The patients, with mean age 45.2±11.5 years and mean BMI 48.6±7.8 kg/m^2, were predominantly Caucasian (70.8%) and female (65.0%). Waist and neck circumference were 135.9±19.3 and 46.7±11.7 cm, respectively. The mean sleep duration was 6.3±1.9 h during weekdays and 6.5±2.0 h during the weekend, suggesting sleep deprivation. Hypertension (40.8%), diabetes mellitus (31.7%), dyslipidaemia (37.8%), and obstructive sleep apnoea (16.0%) were common co-morbidities in our patients. A high proportion of patients were on welfare benefits (55%) and the highest education level achieved was high school level (56.6%). Comfort (53.2%) and boredom (52.4%) eating were quite prevalent while depression (49.2%) and previous bullying (19.5%) were common psychological issues. Approximately a third (29.7%) was taking psychiatric medications. Skipping at least one meal per day (65%), particularly breakfast (63.8%) was common while 70.2% reported eating takeaway meals and 50.4% consuming carbonated drinks regularly. Just over-half reported engaging in some regular physical activity (58.4%), but commonly reported barriers included arthritis (20.7%), back pain (15.7%), and breathlessness (12.4%). The median time spent watching television was 3 h while 1 h was spent on computer use daily. The majority of the patients already had at least one weight loss attempt (71.4%) prior to attending the specialist service. In conclusion, these findings showed that management of extreme obese is complex involving multiple co-morbidities, and psychological and social challenges. Apart from treating cardiovascular risk factors; eating behaviour, psychological issues and barriers on physical state should be supported and addressed.

DOI: 10.1530/endoabs.34.P236

P237

Coffee attenuates induction of insulin resistance in high sucrose-diet fed rats

Ayodele Morakinyo[2], Daniel Adekunbi[1], Kayode Dada[2] & Olufeyi Adegoke[2]
[1]Babcock University, Ilishan-Remo, Nigeria; [2]University of Lagos, Lagos, Nigeria.

Several epidemiological evidences indicate that consumption of coffee is associated with a lower risk of type 2 diabetes mellitus (T2DM); however, there is dearth of experimental data to support these observations. Given that associations do not necessarily infer causality; the present study investigate the effect of coffee consumption on glucose regulation, T2DM and the probable mechanisms of action, if any, using an animal model. Twenty-four (24) Sprague–Dawley rats weighing between 120 and 150 g were randomly divided into four equal groups of six animals. The rats were given either a normal diet (ND); ND supplemented with coffee (ND + COF); high sucrose-diet (HSD); and HSD supplemented with coffee (HSD + COF). Coffee (300 mg/kg BW) was administered by oral gavage once daily while free access to HSD (30% w/v) as drinking water was provided according to the method of Riberio *et al*. (2005) and modifying. Treatment lasted for 12 consecutive weeks during which experimental measurements such as fasting blood glucose (FBG), oral glucose tolerance test (OGTT), and insulin tolerance test (ITT) were performed. Serum insulin level, lipid profile and oxidative parameters were also determined in all experimental rats. HSD rats were insulin resistant and had significantly elevated levels of insulin, malondialdehyde (MDA), triglyceride (TG), LDL while HDL level, superoxide dismutase (SOD) and glutathione (GSH) activities were significantly reduced. In contrast, coffee administration significantly up-regulate glucose tolerance, insulin sensitivity, HDL level, SOD, and GSH activities while MDA, TG, and LDL levels were reduced. These findings suggest that coffee consumption attenuates the development of insulin resistance and/or T2DM in HSD-fed rat.

DOI: 10.1530/endoabs.34.P237

P238

Systemic triglycerides as a key determinant of TLR regulated inflammatory risk in human adipose tissue post bariatric surgical intervention and weight loss

Warunee Kumsaiyai[1], Nasser Al-Daghri[2], Ioannis Kyrou[1], Jana Vrbikova[3], Vojtech Hainer[3], Martin Fried[3], Petra Sramkova[4], Thomas Barber[1,5], Sudhesh Kumar[1], Gyanendra Tripathi[1] & Philip McTernan[1]
[1]Division of Metabolic and Vascular Health, Clinical Sciences Research Laboratories, Warwick Medical School, Coventry, UK; [2]Biomarkers Research Program, Biochemistry Department, College of Science, King Saud University, Riyadh, Saudi Arabia; [3]Institute of Endocrinology, Prague, Czech Republic; [4]OB Clinic, Prague, Czech Republic; [5]Human Metabolism Research Unit, WISDEM, UHCW, Coventry, UK.

Background and aims
Bariatric surgery can lead to a quick reversal in type 2 diabetes mellitus (T2DM) status. However, despite this reversal inflammatory responses may still persist via activation of Toll-like receptors (TLR) within adipose tissue (AT); with triglycerides (TGs) noted as a potential mediator of such inflammation. Therefore the aims of these studies were to understand the impact of TG changes, pre- and post-bariatric surgery, on TLR expression in *ex vivo* AT and the *in vitro* effects of triglyceride rich lipoprotein (VLDL), on TLR expression in isolated human differentiated pre-adipocytes.
Materials and methods
Obese, T2DM, female subjects (age: 54.6 ± 6.6 years, BMI pre (41.2 ± 5.5 kg/m^2) and 6 months post-surgery (36.05 ± 5.16 kg/m^2; $n = 30$) underwent bariatric surgery (banding ($n = 8$); plication ($n = 14$); and biliopancreatic diversion ($n = 8$)). Biochemical data and abdominal subcutaneous AT (AbdSc AT) samples were taken during surgery and 6 months post-surgery. Real-time PCR assessed TLR expression. Human differentiated pre-adipocyte Chub S7 cells were used to examine transcriptional effects of VLDL on TLR expression.
Results
Following surgical intervention, BMI ($P < 0.001$), blood glucose ($P < 0.001$), insulin ($P < 0.001$), HOMA-IR ($P < 0.001$), TG ($P < 0.05$), cholesterol ($P < 0.001$), and LDL-cholesterol ($P < 0.05$) were significantly improved. There was a significant reduction in TLR4 mRNA post-surgery ($P < 0.01$) irrespective of surgery type. It was also noted that subjects with the greatest drop (55.5% reduction) in TGs post-surgery ($P < 0.001$) showed a significant correlated reduction in TLR4 mRNA expression ($P < 0.001$). *In vitro* treatment of differentiated Chub S7 cells highlighted VLDL induced TLR4 mRNA expression ($P < 0.05$).
Conclusion
There is a reduction in AT inflammation as denoted by TLR expression. The reduction in AT inflammation appears dependent on how successfully subjects reduce their serum triglyceride, which is supported by *in vitro* studies. These studies suggest that bariatric surgery lead to metabolic improvement with weight loss, whilst dietary intervention is still required to ensure TGs reduce to reduce inflammation.

DOI: 10.1530/endoabs.34.P238

P239

Upregulation of AKR1C3 expression by insulin in a human differentiated preadipocyte cell line

Philip House, Michael O'Reilly, Laura Gathercole & Jeremy Tomlinson
University of Birmingham, Birmingham, UK.

Polycystic ovary syndrome (PCOS) is a clinical triad of anovulation, hyperinsulinaemia, and androgen excess. Adipose androgen generation of testosterone from androstenedione by aldo-ketoreductase type 1C3 (AKR1C3) may contribute to hyperandrogenism. We hypothesised that insulin may upregulate adipose AKR1C3 expression *in vitro* in a human differentiated preadipocyte cell line.
We analysed AKR1C3 expression in the SGBS human preadipocyte cell line. Preadipocytes were differentiated into adipocytes under optimised conditions over 14 days. Differentiated adipocytes were exposed to increasing concentrations of insulin, androstenedione, and testosterone in serum-free media for 20 h. We used real-time quantitative PCR to determine gene expression in SGBS cells across differentiation (days 0, 7, and 14) and after acute insulin treatment. Experiments were performed in triplicate. Data are expressed as mean ± S.E.M. for delta *C*T (d*C*T).
Markers of adipogenesis PPARγ and LPL increased significantly across differentiation. Expression of AKR1C3 also increased across differentiation (dCT day 0, 13.3 ± 0.1; day 14, 7.9 ± 0.9, $P = 0.01$). Acute insulin treatment significantly increased gene expression of AKR1C3 in a dose-dependent manner (insulin 10 mM, dCT 7.3 ± 0.1; insulin 20 mM, dCT 6.5 ± 0.1, $P < 0.05$ for both compared to control, $P = 0.02$ between treatments). Co-incubation of insulin with androstenedione and testosterone significantly reduced AKR1C3 expression compared to insulin alone, $P = 0.03$ and $P = 0.01$ respectively.
AK1C3 expression in a differentiated human preadipocyte cell line is upregulated by insulin. Adipose tissue may be a key site of cross-talk between insulin signalling and androgen metabolism. Local adipose androgen generation in PCOS may be increased in hyperinsulinaemic conditions, and represent a possible target for therapeutic intervention.

DOI: 10.1530/endoabs.34.P239

P240

The outcome results in patients with type 2 diabetes mellitus and obesity after either adjustable gastric banding or Roux-en-Y gastric bypass

Andrzej Rys & Tabinda Dugal
Department of Endocrinology and Diabetes, Royal Cornwall Hospital, Truro, UK.

The prevalence of the type 2 diabetes and obesity are on the rise globally. Initial interventions for these groups of patients remain diet, exercise and medications. If these measures are insufficient gastrointestinal surgery offers a very good alternative for obesity and type 2 diabetes treatment.
We report the outcome results for patients who underwent either adjustable gastric banding (AGB) or Roux-en-Y (RNY) gastric bypass in the years 2009–2012.

Out of 33 patients (Seven men, 26 women, average age 48.4 years), 11 underwent AGB and 22 had RNY. Preoperatively there were no statistically significant differences in: weight, excess of weight, BMI, HbA1c, blood pressure between AGB and RNY subgroups.

In the AGB subgroup the following results were obtained 6 months after the operation: average loss of weight (LOW) 10.87 kg, 18.18% achieved 50% estimated weight loss (EWL), and 0% achieved 70% EWL. We observed HbA1c reduction of 5.66 mmol/mol.

12 months after the operation average LOW was 14.8 kg, 9.09% achieved 50% EWL, 0% achieved 70% EWL. We observed HbA1c reduction of 7.41 mmol/mol and reduction in BP of 9.6/5.6 mmHg.

In the RNY subgroup 6 months after operation the results were as follows: average LOW 30.9 kg, 71.43% achieved 50% EWL, and 23.81% achieved 70% EWL. We observed HbA1c reduction of 24.1 mmol/mol.

12 months after the operation average LOW was 39.95 kg, 100% achieved 50% EWL, and 58.33% achieved 70% EWL. We observed HbA1c reduction of 13.27 mmol/mol. We observed overall reduction in BP 12.5/4.95 mmHg

The results show significantly better achievement of EWL and reduction in HbA1c in the RNY subgroup. These results were more sustainable in RNY group 12 months after the operation. Our report supports the more favourable outcomes in patients undergoing RNY gastric bypass procedures.

DOI: 10.1530/endoabs.34.P240

P241

Diabetes in pregnancy and birth weight: differential effects due to ethnicity in a real-life observational study

James Wilson[1], Hema Venkataraman[2], Jessica Cheong[4], Nithya Sukumar[2], Kamlesh Khunti[3] & Ponnusamy Saravanan[2]
[1]Barnet Hospital, London, UK; [2]Warwick Medical School, University of Warwick, Coventry, UK; [3]Department of Health Sciences, University of Leicester, Leicester, UK; [4]William Harvey Hospital, Ashford, UK.

Background

Macrosomia (BW >4.5 kg) is a well-recognised complication of maternal hyperglycaemia and is associated with poor maternal and foetal outcomes. Traditional risk factors of macrosomia do not fully explain all variation as it can happen in normoglycaemia and in well-controlled diabetes. The relative contributions of these factors in different ethnicities are unknown. We investigated the predictors of BW in South Asian (SA) and White Caucasian (WC) pregnancies, and the risk of macrosomia.

Method

Data from 88 606 singleton births across Leicester were collected (1994–2006). Multivariate linear and logistic regression models are used to analyse the predictors of BW in full-term births. Ethnicity effects were determined using the Mann–Whitney- U (categorical) and χ^2 tests (continuous).

Results

53 128 births had complete dataset (WC: 44 657 (84.1%); SA: 8471 (15.9%)). The mean BWs were: WC: 3445 ± 488 g; SA: 3102 ± 452 g; $P<0.001$. Macrosomia rates were: WC: 12.6%; SA: 2.9% (odds ratio (OR): 0.21, 95% CI: 0.18, 0.24; $P<0.001$). Maternal diabetes ($n=837$) prevalence was higher in SA (3.47 vs 1.22%; OR: 2.92, 95%CI: 2.53,3.37; $P<0.001$). Adjusting for all covariates, higher gravida, non-smoking, low deprivation, maternal age and BMI, gestational age, and male-sex, independently and positively predicted BW and macrosomia. Blood pressure ($n=36\,697$) was not independently associated with BW. Babies born to WC mothers were heavier (male: 291.2 g, 95% CI: 276.7, 305.7; female: 254.2 g, 95% CI: 239.5, 268.9 g; $P<0.001$) compared to SA babies. Maternal diabetes contributed differentially to BW within different ethnicities (WC: 284.5 g (95% CI: 248.1, 320.9 g) vs SA: 213.7 g (95% CI: 164.0, 263.4 g), $P=0.024$).

Conclusion

Maternal diabetes is a strong independent risk factor for macrosomia and the magnitude of weight gain is significantly different between WC and SA. After correcting for all variables, the weight gain due to WC pregnancies is greater than that for SA pregnancies. This result may reflect different levels of care between ethnicities or may be the effect of genetic variation. Further research is required to assess the interaction of ethnicity on deprivation, poor engagement and/or poor compliance with treatment.

DOI: 10.1530/endoabs.34.P241

P242

Evolutionary conserved novel targets of NGN3, located on the short arm of chromosome 20, may have a role in the development of the endocrine pancreas

Oluwafikunayo Orekoya[1,2], Andrew Berry[2] & Neil Hanley[2]
[1]Manchester Medical School, University of Manchester, Manchester, UK; [2]Faculty of Medical and Human Sciences, Institute of Human Development, Centre for Endocrinology and Diabetes, University of Manchester, Manchester, UK.

Background/aims

The transcription factor neurogenin 3 (NGN3) is required for pancreatic islet cell specification from multipotent progenitor cells; yet we have limited understanding of the downstream genetic program it directly initiates. A 3 Mb centromeric region on chromosome 20p contains a number of genes activated downstream of NGN3 including *INSM1* and *NKX2.2*. We proposed that NGN3 alters the expression of other genes within this region as potential downstream targets.

Methods

In-silico analysis of the 3 Mb genomic region containing *INSM1* and *NKX2.2* was carried out to identify potential target genes and search for putative NGN3 binding elements (E-boxes). An engineered human pancreatic ductal cell line, PANC1, with inducible *NGN3* expression (Piper Hanley *et al*. *J Endo*, 2010) was used to up-regulate NGN3 following which quantitative RT-PCR (qRT-PCR) analysed gene expression.

Results

NGN3 was induced in the PANC1 cell line by infection with recombinant adenoviral vectors expressing the human *NGN3* following which eight evolutionary conserved genes were identified by qRT-PCR to be significantly altered in expression. *AF147354, MIR3192, LINC00261*, and *THBD* were down-regulated by at least 0.27-fold by NGN3 whereas *INSM1, RIN2, C20orf74*, and *PLK1S1* were up-regulated by at least 0.35-fold, making them all potential NGN3 targets. Evolutionary conserved elements consistent with putative E-boxes were identified upstream of *AF147354, LINC00261, INSM1, RIN2, C20orf74*, and *PLK1S1*.

Discussion

The thrombomodulin gene (*THBD)* and polo-like kinase 1 substrate 1 gene (*PLK1S1)* were the two genes most significantly affected by *NGN3* induction, with a 1.2- and 0.5-fold change respectively. *PLK1S1* up-regulation may be important in stabilising and strengthening the expanded pericentriolar region of replicated chromosomes; whilst *THBD* down-regulation may enhance cell migration, an important aspect of islet formation during human pancreas development. In summary, both *PLK1S1 and THBD* could be novel targets of NGN3 and may play a role in islet cell development.

DOI: 10.1530/endoabs.34.P242

P243

Decreased brain 11β-HSD1 expression following inflammation; a role in regulating brain energy homeostasis?

Manu Verma[1], Zhenguang Zhang[1], Annie Mackellar[2], Jonathan Seckl[1], Megan Holmes[1] & Karen Chapman[1]
[1]University/BHF Center for Cardiovascular Sciences, University of Edinburgh, Edinburgh, UK; [2]MRC Centre for Inflammation Research, University of Edinburgh, Edinburgh, UK.

Chronically elevated glucocorticoid (GC) levels alter cognition and increase cardio-metabolic disease risk. Negative feedback suppression of the hypothalamic–pituitary–adrenal (HPA) axis, including at the hippocampus, maintains low/basal circulating GC levels. Intracellular GC can be increased, without alteration in circulating levels, by the activity of 11β-hydroxysteroid dehydrogenase type1 (11β-HSD1). In some tissues, 11β-HSD1 expression is increased by pro-inflammatory cytokines and by GC themselves. We hypothesized that 11β-HSD1 in the brain will be regulated during inflammation by pro-inflammatory cytokines and/or GC and will modulate local inflammation induced effects on HPA axis activity and energy homeostasis. To investigate whether 11β-HSD1 influences brain metabolism basally and whether 11β-HSD1 in the hippocampus and/or the hypothalamus is regulated during inflammation, levels of mRNA encoding enzymes and transporters relevant to energy homeostasis were quantified in whole brain of 11β-HSD1−(KO) and C57BL/6 (WT) mice by qPCR. Inflammation was induced in an experimental model of arthritis (i.p. injection of arthritogenic K/BxN serum) or lung inflammation (i.t. administration of *Staphylococcus aureus*). 11β-HSD1 mRNA levels were quantified at day15 (K/BxN arthritis) and 24 h post inoculation (*Staphylococcus aureus*). Lactate transporter, *Mct1*, mRNA levels were higher in KO (1.3-fold; $P<0.05$)

suggesting increased lactate uptake. Levels of *Pfk-1* and *Ldh-b* mRNA (encoding glycolytic enzymes) were lower in KO (0.75– to 0.82-fold; $P<0.05$) suggesting increased glycolysis. KO had higher mitochondrial DNA content and mRNA encoding mitochondrial enzymes, *Cs* and *Cox6c* (1.3–,1.2 to 1.3-fold, respectively; $P<0.05$) suggesting increased mitochondrial number. Arthritis or lung inflammation increased hippocampal and hypothalamic *Tnf-α* mRNA levels ($P<0.05$), reduced hippocampal 11β-HSD1 mRNA levels ($P<0.001$) with a similar, though non-significant trend, in hypothalamus. These data suggest that 11β-HSD1 deficiency increases glycolysis and energy substrate (lactate) in brain. Following inflammation, 11β-HSD1 mRNA levels are reduced in brain, in contrast to the up-regulation previously described in peripheral tissues suggesting a possible mechanism to increase brain glycolysis in response to inflammation.

DOI: 10.1530/endoabs.34.P243

P244

Capillary blood glucose monitoring and ambulatory peritoneal dialysis: reassuring the patient

Waiel Bashari & Samson Oyibo
Peterborough City Hospital, Peterborough, UK.

Introduction
Icodextrin is a large molecular weight glucose polymer used in the diasylate called Extraneal. It is hydrolysed to oligosaccharides, which are read as glucose by some blood glucose monitoring (BGM) devices resulting in overestimation of capillary blood glucose (CBG) readings. This is dangerous for patients on ambulatory dialysis trying to achieve optimum diabetes control.
Case
A lady with type 1 diabetes using an insulin pump for diabetes control while on ambulatory peritoneal dialysis for end-stage renal disease, had concerns about overestimation of CBG readings by her Contour BGM device and test strip. She used extraneal by day and a glucose-containing dialysate by night. Fear of hypoglycaemia made her keep CBG levels above 14 mmol/l, even though the Contour BGM test strips are noted as safe in such patients. We aimed to reassure her.
Method
She provided CBG readings (pre-breakfast, post-breakfast, pre-lunch, and post-lunch, late afternoon) using her Contour BGM device (glucose dehydrogenase flavin-adenine dinucleotide method). Paired blood samples were also analysed using the One Touch Ultra BGM device (glucose oxidase method) and our laboratory (enzymatic hexokinase method). Agreement with the laboratory method was acceptable if at least 95% of analyser values did not deviate by more than 20%.
Results
Table 1 shows the CBG values from both BGM devices and the corresponding laboratory values. Graphical representation demonstrated that none of the CBG values from both BGM devices deviated by more than 20% from the corresponding reference laboratory values.

	Lab value hexokinase method (mmol/l)	**Contor BGM** (mmol/l)	**One Touch Ultra BGM** (mmol/l)
Pre-breakfast	23.3	22.0	25.8
Post-breakfast	21.4	21.6	22.3
Pre-lunch	10.8	10.2	11.4
Post-lunch	9.5	9.0	10.4
Late afternoon	5.4	5.6	6.4

Conclusion
The Contour BGM device estimated CBG levels accurately when compared to the reference laboratory method and another BGM device: it was unaffected by the Icodextrin-containing dialysate. Her hyperglycaemia was true hyperglycaemia from the glucose-containing dialysate used overnight. She was reassured and could make appropriate changes to her basal rate of insulin with confidence.

DOI: 10.1530/endoabs.34.P244

P245

Case report: the role of somatostatin analogue therapy in nesidioblastosis following Roux-en-Y bypass surgery

Rajamani Manikandan, Shiva Mongolu & Azraai Nasruddin
University Hospital Southampton, Southampton, UK.

Background
Postprandial hyperinsulinemic hypoglycaemia due to nesidioblastosis is a significant and debilitating complication after Roux-en-Y gastric bypass surgery (RYGB). There is growing evidence suggesting this is due to increased incretin hormones secretion. Nesidioblastosis can be difficult to manage with variable response to different pharmacological therapies and some requiring partial/total pancreatectomy or revision of bypass surgery pouch. We present a patient who has had partial response to octreotide.
Case report
A 45-year-old lady had RYGB 18 months ago for significant reflux symptoms. She also lost 35 kg but developed severe postprandial neuroglycopenic symptoms that occurred 90–120 min after meals. She gained 10 kg. During oral glucose tolerance test (OGTT) her blood glucose dropped to 1 mmol/l at 90 min with insulin 11.1 μu/l and C-peptide 2595 pmol/l. She did not have any benefit from dietetic advice, low GI diet, acarbose, and Guar gum. Pancreatic CT scans and calcium stimulation test did not localize a cause for her hyperinsulinemia. Her symptoms improved partially with diazoxide but it was withdrawn due to side effects. Repeating OGTT on diazoxide, her glucose dropped to 1.4 mmol/l in 90 min with peak insulin 158.1 μu/l and peak C-peptide 5826 pmol/l. Her symptoms improved with octreotide 50 μg TDS. OGTT done after 6 months on octreotide therapy showed peak glucose of 14.9 mmol/l at 90 min with blunted insulin response with peak levels of 33.5 μu/l. However, she continued to report intermittent postprandial hypoglycaemia. We switched her to octreotide LAR but hypoglycaemia got worse.
Discussion
Our case highlights the difficulties in managing the symptoms of nesidioblastosis. Subcutaneous octreotide worked well initially but the effects became attenuated. Her symptoms could be episodes of rebound hypoglycaemia when octreotide effect wore off. It is unclear why octreotide LAR did not work. Pasireotide with its potent suppression of incretin release is likely to work well. We have obtained funding for a trial of pasireotide therapy.

DOI: 10.1530/endoabs.34.P245

P246

Impact of chronic heart failure on adipose tissue functional plasticity: a role for fatty acids?

Andrew Yale[1], Malcolm Cobb[1], Alexander Lyon[2] & Alison Mostyn[1]
[1]School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington, Leicestershire, UK; [2]NIHR Cardiovascular Biomedical Research Unit, Royal Brompton Hospital, London, UK.

Chronic heart failure (HF) is a major cause of morbidity and mortality in humans and domestic animals. HF is characterised by chronic inflammation and functional changes in non-cardiovascular tissues; obesity is also associated with low level chronic inflammation, with adipose tissue (AT) exhibiting infiltration of macrophages and alterations in fatty acid (FA) profiles. The aim of this project was to investigate expression of regulators of lipid metabolism and inflammation and fatty acid profiles in AT from a rodent model of HF.
Rats with HF post myocardial infarction were compared with age matched non-failing controls (C)[1]. Intra-abdominal (IA) and subcutaneous (SC) adipose tissue depots were sampled after humane euthanasia for real-time PCR (QPCR) analysis of stearoyl-CoA desaturase-1 (SCD1), interleukin 1 (IL1) and phosphoenolpyruvate carboxykinase (PCK1). Gas chromatography was used to generate FA profiles and tissue triglyceride (TAG) content was assessed by colourimetric assay. Statistical significance was assessed using ANOVA with *post hoc* Tukey tests.
In healthy animals SCD1 was significantly lower in SC compared to IA. HF-IA adipose tissue also demonstrated a significant reduction when compared to C-IA (C-IA 1.4 ± 0.06; C-SC 1.0 ± 0.03; and HF-IA 1.17 ± 0.08, $P<0.05$). PCK was higher in SC compared to IA in control rats (C-IA 0.7 ± 0.05; and C-SC 1.0 ± 0.03, $P<0.05$), but there was no effect of HF. TAG was significantly higher in HF-IA compared to both C-IA and HF-SC tissue (C-IA 56.8 ± 27.2; HF-IA 199.8 ± 15.4; and HF-SC 111.3 ± 20.6 mg/g, $P<0.05$). FA analysis identified differences in several species; including reductions in C10, C15 and C17 in

HF-SC; however, most interesting was a reduction of both C20:5n3 (EPA) and C22:6n3 (DHEA) in HF-SC.
Despite a reduction in SCD1 expression, we did not detect any difference in mono-unsaturated FA species in the HF rats. EPA and DHEA are essential omega 3 FAs with recognised anti-inflammatory and cardio-protective effects; a reduction in HF suggests altered uptake and metabolism in these animals.
AYale was supported by a Society for Endocrinology Summer Studentship
Reference
1. Lyon AR, *et al*. Circulation: arrhythmia and electrophysiology. 2011 **4** 362.
DOI: 10.1530/endoabs.34.P246

P247

Maternal metabolic adaptations in pregnancy are associated with altered circadian rhythmicity

Georgia Papacleovoulou[1,2], Vanya Nikolova[1,2], Olayiwola Oduwole[2], Malcolm Parker[2] & Catherine Williamson[1,2]
[1]King's College London, London, UK; [2]Imperial College London, London, UK.

Introduction
Pregnancy is associated with maternal metabolic adaptations (increased cholesterol and triglycerides) that are essential for the development and maintenance of the fetus. Physiological and behavioural changes are driven via biological clocks entrained by the light–dark/rest–activity cycles that define feeding time and body temperature. A number of oscillators are present in the peripheral organs that are synchronised by cues from the suprachiasmatic nucleus. The core clock machinery drives rhythmic metabolic activities and receives feedback from intracellular metabolites.
Hypothesis
Maternal metabolic adaptations in pregnancy are a result of altered circadian rhythmicity of metabolism-associated genes.
Methods
C57BL6 mice were sacrificed in early pregnancy (day 7 post-coitum (pcm)-pre-placentation) and in the third trimester of pregnancy (day 14 pcm) at 4 h intervals over a 24 h light/dark cycle. Mice on day 2 pcm were used as controls.
Results and conclusions
Circadian rhythmicity of hepatic lipogenic genes (Fas, Scd-1, Scd-2, Lpl, and Hmgcr) was increased on day 7 pcm with a peak expression during the night and was blunted on day 14 pcm mice compared to controls. In gonadal fat of pregnant animals, there was a loss in circadian rhythmicity of lipogenic (Fas) and lipolytic (Hsl, Cd36, and Hadh) genes throughout pregnancy. In skeletal muscle, we demonstrated loss of rhythmicity of fatty acid oxidation-related genes (Cpt1β, Fatp1, and Glut4) on day 7 pcm that was restored on day 14 pcm with a peak expression during the light phase. We showed circadian rhythmicity in placental targets involved in lipid transport (Lxrβ, Abca1, Ldlr, pFabp-pm, and Fatp4) with a peak expression in the dark phase on day 14 pcm. These data suggest altered syncronisation of metabolic tissues at different stages of pregnancy. A shift from fat storage in early pregnancy (driven by a rhythmic liver) to energy mobilisation later in pregnancy (driven by a rhythmic muscle) appears essential for development and maintenance of the growing fetus.
DOI: 10.1530/endoabs.34.P247

P248

Abstract Withdrawn.

DOI: 10.1530/endoabs.34.P248

P249

Derivatisation of estrogens enhances specificity and sensitivity of analysis by liquid chromatography tandem mass spectrometry

Abdullah M M Faqehi[1], Diego F Cobice[1], Gregorio Naredo[2], Tracy C S Mak[1], Brian R Walker[1,2], Natalie Z M Homer[2] & Ruth Andrew[1,2]
[1]Endocrinology, Queen's Medical Research Institute, University/British Heart Foundation Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK; [2]Mass Spectrometry Core, Wellcome Trust Clinical Research Facility, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, UK.

Physiological circulating concentrations of estrogens are very low in men and postmenopausal women (<50 pg/ml), which presenting analytical challenges. Immunoassays can detect as low as 30 pg/ml, but cross-reactivity is a concern. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) offers greater specificity than immunoassays, but ionisation of estrogens is inefficient. Derivatisation, which introduces charged moieties, may enhance ionisation. Dansylated derivatives of estrogens have been reported previously but these generate non-specific product ions originating from the "reagent" group in MS/MS. Therefore, a derivative with product ions specific to individual estrogens was sought.
Estrogens were extracted from human plasma using solid phase extraction and derivatised using 2-fluoro-1-methylpyridinium-*p*-toluenesulfonate (FMP). Estrogen FMP derivatives were quantified using triple quadrupole MS in positive electrospray ionisation mode, following LC separation. Reaction conditions were optimised and the assay validated for linearity, precision, accuracy, and stability. Limits of detection and quantitation (LOD and LOQ) were evaluated.
Transitions for the FMP derivatives of estrone (E_1) and estradiol (E_2) were compound specific (m/z $362 \rightarrow 238$ and m/z $364 \rightarrow 110$ respectively). The LOD was 2 pg/ml and the method was linear across the range 5–200 pg/ml. The values of intra- and inter-assay variables were acceptable at LOQ (5 pg/ml), precision (intra): 12%, 11% (inter): 15%, 13%; accuracy (intra): $\pm 18\%$, $\pm 17\%$, (inter): $\pm 19\%$, $\pm 19\%$ for E_1, E_2 respectively. The derivatives demonstrated suitable stability over 24 h, (7–9% degradation). Using this approach, E_1 and E_2 were detected in human plasma (0.5 ml female, 1 ml male) at levels (7–9), (55–59) pg/ml in males and (78–84), (204–216) pg/ml in females for E_1 and E_2 respectively with signal to noise of 5–10.
Here, we have demonstrated that FMP derivatisation in conjunction with LC–MS/MS is suitable for quantitative analysis of low abundance estrogens in biological fluids. The assay offers clear advantages in specificity and sensitivity over immunoassay and existing MS techniques for estrogens analysis.
DOI: 10.1530/endoabs.34.P249

P250

Virally delivered target-specific optogenetic stimulation of PPG neurons in the nucleus of the solitary tract

James E Richards[1], Simon C Cork[1], Marie Holt[1], Frank Reimann[2], Fiona M Gribble[2] & Stefan Trapp[1]
[1]UCL, London, UK; [2]Cambridge Institute for Medical Research, Cambridge, UK.

Glucagon like peptide-1 (GLP-1) is derived from selective cleavage of the preproglucagon (PPG) molecule synthesised in intestinal L-cells. The physiological action of GLP-1 is most commonly recognised as a peripherally released incretin, but a subset of neurons in the lower brainstem, the PPG neurons, also express GLP-1. The majority of PPG neuronal cell bodies are located in the nucleus of the solitary tract and their axons project to numerous sites throughout the CNS. Many of these are located in regions of the forebrain associated with food intake. We have combined the use of a CRE recombinase-dependent adeno-associated virus, a transgenic mouse line, which expresses tdRFP and CRE under control of the PPG promoter and precise stereotaxic injection to target these neurons using optogenetics. We demonstrate successful and high-level expression of Channelrhodopsin2-eYFP (ChR2) selectively in tdRFP expressing PPG neurons located in the caudal brain stem but also in axons projecting from these neurons to the forebrain. Electrical activity of PPG neurons in acute slices, expressing ChR2, was analysed with whole-cell patch-clamp recordings. In current clamp, laser light stimuli at 455 nm of 20 ms duration reliably elicited action potentials in PPG neurons, with shorter light pulses (10–2 ms) causing smaller depolarisations and decreased probability of eliciting action potentials. In voltage clamp at a holding potential of -60 mV, inward currents of $\sim$ 70 pA amplitude were elicited by light pulses of 10 ms or longer and were sustained for

the duration of the light pulse. Shorter light pulses did not reach full amplitude. These results demonstrate that selective optogenetic stimulation of the PPG neurons can be achieved by targeting these neurons with Cre-dependent virally-encoded ChR2 expression, and will allow investigation of their involvement in feeding and cardiovascular function.

DOI: 10.1530/endoabs.34.P250

P251

Metformin and acute kidney injury: is it the culprit?

Phey Shen Lee & Jonathan Donald Mackay
Blackpool Victoria Hospital, Blackpool, UK.

Three people (two males), with mean age (range) 65 (49–75) years, presented as emergencies with acute renal failure (AKI). They had type 2 diabetes mellitus, with mean duration 7 (2–12) years. All three were taking anti-hypertensive therapy: two were on ramipril and one on losartan and indapamide. All three were taking metformin with mean daily dose 2.6 (1.7–3.0) g. All three patients had mild renal impairment (CKD stage 3), but renal function was stable 2–3 months before presentation with mean blood urea 8.1 (5.6–9.5) mmol/l, creatinine 118 (108–133) μmol/l and eGFR 49 (43–59) ml/min per l. They presented with a 1 week history of symptoms including vomiting (2), diarrhoea (2), unsteadiness (3), confusion (1), and falls (2). On admission the mean blood glucose was 8.5 (4.9–13.0) mmol/l, urea 32 (22 – 42) mmol/l, and creatinine 763 (652–978) μmol/l. They were acidotic with mean pH 7.19 (7.15–7.23), bicarbonate 15.6 (10.3–21.2) mmol/l, and lactate 3.6 (2.0–5.9) mmol/l. The mean blood metformin level was raised at 12.2 (11.0–14.3) mg/l. The accepted therapeutic range is 0.5–2.0 mg/l). Metformin, ramipril, losartan and indapamide were discontinued. One patient required haemofiltration but in all three urine output soon improved with i.v. fluid therapy. Renal function was back to baseline 2–3 months later with mean blood urea 7.5 (6.8–8.4) mmol/l, creatinine 113 (104–131) μmol/l and eGFR 52 (45–60) ml/min per l.

Metformin is eliminated, unchanged, by renal excretion. We suggest that inappropriately high metfomin doses in these patients with mild renal impairment has led to escalating blood metformin levels, which in turn largely accounted for the presenting symptoms and nephrotoxicity. However, the AKI was reversible with withdrawal of metformin and supportive therapy.

Metformin should be better recognised as a potential cause of AKI. Doses should be reduced, probably halved, in CKD stage 3; and metformin avoided altogether in CKD stage 4.

DOI: 10.1530/endoabs.34.P251

P252

The role of glucocorticoid metabolism in bile acid homeostasis

Monica Naomi Opiyo[1], Geneviève Escher[2], Christopher Kenyon[1], Jonathan Seckl[1], Ruth Andrew[1] & Karen Chapman[1]
[1]Edinburgh University, Edinburgh, UK; [2]Departement für Nephrologie, Hypertonie und klinische Pharmakologie, Kinderspital, Freibursgtr, Switzerland.

Background and aims

Bile acids are conserved through enterohepatic circulation, a glucocorticoid-modulated process. 11β-Hydroxysteroid dehydrogenase type-1 (11β-HSD1) converts cortisone/11-dehydrocortisone to cortisol/corticosterone, thus increasing intracellular glucocorticoid levels. 11β-HSD1 also metabolises 7-oxo-lithocholic acid, a bile acid. 11β-HSD1 is highly expressed in the liver and may alter bile acid transport through regeneration of active glucocorticoids or may directly metabolise bile acids, thus altering profile. We compared bile acid synthesis, release, their enterohepatic circulation and profile in *Hsd11b1*$^{-/-}$ and isogenic C57Bl/6 control mice.

Methods

Adult male, chow-fed mice (eight per group) were fasted for 4 h or fasted for 4 h then re-fed 4 h. Serum, and liver and gall-bladder bile acid concentrations and profiles were measured by spectrophotometry/gas chromatography–mass spectrometry.

Results

Fasted *Hsd11b1*$^{-/-}$ and C57Bl/6 mice had similar volumes of bile (3.2 vs 5.0 l). 7β-Hydroxylated acids (ω-muricholic > β-muricholic > ursodeoxycholic acid > others) predominated in bile of C57Bl/6 mice while 7α-hydroxylated acids (cholic > α-muricholic > chenodeoxycholic acid > others) predominated in *Hsd11b1*$^{-/-}$ mice; the ratio of 7α:7β acids was >100 greater in *Hsd11b1*$^{-/-}$ mice. In fasted *Hsd11b1*− mice, bile acid concentrations were higher in serum (*Hsd11b1*$^{-/-}$: 30 ± 9 vs C57Bl/6: 3.8 ± 1.3 nM, $P < 0.0001$) and liver (*Hsd11b1*$^{-/-}$: 243 ± 18 vs C57Bl/6: 139 ± 19 nmol/g, $P < 0.001$). Re-feeding caused gall bladder emptying in C57Bl/6 mice (49.6 ± 8% of fasted weight, $P < 0.0001$) and increased serum (62.5 ± 7.6 nM) and liver (497 ± 20 nmol/g) bile acid concentrations, while in *Hsd11b1*− mice, the gall bladder did not empty (92 ± 7.8% fasted weight) and serum (24 ± 12 nM) and liver (122 ± 21 nmol/g) bile acid concentrations were similar to the fasted state. Conclusion: Bile acid release and the pattern of synthesis is controlled by 11β-HSD1 activity in mice. This may affect digestion and, depending on functional differences of 7α- and 7β- hydroxylated bile acids, may have significant effects on lipid and sterol metabolism and bile acid-mediated transcription.

DOI: 10.1530/endoabs.34.P252

P253

Gitelman syndrome

Veneranda Lorelei Salazar, Komal Imtiaz, Tamsin Rimmer & Arwa Amijee
Lancashire Teaching Hospitals, Chorley, Lancashire, UK.

A 61-year-old female was referred after being found to be hypokalemic and hypomagnesemic on routine blood test by her GP. Her low potassium of 2.7 has been persisting in spite of treatment with Kay-Cee-L. She denies using laxatives, diuretics, or excess liquorice. She reports intermittent episodes of diarrhea and nocturnal cramps. On examination, she was noted to be hypertensive. A full hormonal profile including 5 HIAA, 24 h urine catecholamines and coeliac screen were negative. Colonoscopy was normal. Her plasma renin was elevated with a normal aldosterone: renin ratio. A CT scan of her abdomen to rule out adrenal adenoma was also normal.

She was referred to a renal physician to rule out renal tubular defect. Her 24 h urine excretion was 115 mmol/24 h with serum potassium of 3.0 showing inappropriate urine potassium wasting in the context of hypokalemia. Gitelman syndrome was considered and molecular testing was done. The diagnosis of Gitelman's syndrome was confirmed when SLC12A3 mutations were detected. She was commenced on Amiloride and carrier testing was done on her sister.

Gitelman syndrome is an autosomal recessive renal tubular disorder characterized by hypokalemic metabolic alkalosis with hypocalciuria and hypomagnesemia. It is caused by loss of function of sodium–chloride symporter in the distal convoluted tubule and by the presence of the homozygous mutation in the SLC12A3 gene. Patients may complain of mild muscular cramps or weakness expressed as fatigue or irritability. Tetany and paralysis have also been reported. The surreptitious abuse of diuretics (or laxatives) remains the commonest differential diagnosis.

The treatment can be difficult, as the degree of salt wasting may be severe. Aggressive replacement of salt, and potassium in particular, is essential. Some patients respond well to the administration of indomethacin, especially in type II Bartter syndrome. Magnesium supplementation is also a useful adjunct.

DOI: 10.1530/endoabs.34.P253

P254

Abstract Withdrawn.

DOI: 10.1530/endoabs.34.P254

P255

Serum ferritin levels are correlated with total adiposity, but not BMI in obese males

M Gulrez Zariwala, Ania Kosicka, Richard Mackenzie & Derek Renshaw
University of Westminster, London, UK.

According to the World Health Organisation iron deficiency is the most prevalent form of under-nutrition worldwide. Historically, the causes of iron deficiency were related principally to nutritional deficiencies and this remains a significant factor in many parts of the developing world. However, obesity is also a known cause of iron deficiency and is increasingly correlated with altered iron status and iron deficiency in affected individuals. As the obesity epidemic spreads to developing countries, iron deficiency caused by obesity is likely to increase in incidence. Altered iron status in obese individuals has been reported previously in many studies involving children, adolescents, and adult females. However, using BMI as a measure of adiposity in previous studies revealed no significant correlation with iron status in adult males.

We recruited 105 healthy, non-diabetic males in a cross sectional human study to correlate iron status against BMI, total adiposity –% body fat (%BF) and waist:hip ratio (waist:hip). BMI ($R=0.807$, $P<0.001$), %BF ($R=0.848$, $P<0.001$) and waist:hip ($R=0.706$, $P<0.001$) all significantly correlated with plasma leptin levels. BMI ($R=0.458$, $P<0.001$), %BF ($R=0.336$, $P<0.001$) and waist:hip ($R=0.382$, $P<0.001$) also correlated with the acute phase protein CRP, which is known to be raised in obesity. Serum ferritin was also measured in this population, but did not significantly correlate with BMI or waist:hip. However, serum ferritin significantly correlated with %BF ($R=0.258$, $P=0.008$), and was also correlated with plasma insulin ($R=0.342$, $P<0.001$).

This data suggests that the use of %BF may be a more sensitive measure of adiposity than BMI in studies investigating iron status–particularly in obese males. The data further suggest a causative link between obesity, altered iron status, and insulin resistance in obese non-diabetic humans.

DOI: 10.1530/endoabs.34.P255

P256

Ventricular dysfunction in the new born secondary to maternal vitamin D deficiency

Sampath Satish Kumar & Akshatha Taranath Kamath
Narayana Hospitals, Bangalore, Karnataka, India.

Introduction

The prevalence of vitamin D deficiency has been increasing worldwide. This can be due to inadequate exposure to sunshine, dietary deficiency, dark skin, covering the whole body due to religious custom. Here, we present an interesting case of a newborn baby with severe hypocalcemia developing seizures and severe cardiac problems secondary to maternal vitamin D deficiency.

Case history

A newborn male baby was admitted to cardiac ITU with a history of severe biventricular dysfunction (LVEF 35–40%), symptomatic treatment started with ionotrophic support. Initial investigation revealed PTH 10.4 pg/ml (11.1–79.5), ionized calcium 2.6 mg/dl (4.5–5.3), corrected calcium 5.5 mg/dl (7.6–11.3), vitamin D 8.31 ng/ml (30–100) and magnesium 1.1 mg/dl (1.3–2.7). Baby been started on calcium infusion, calcium syrup and capsule calcitriol. Corrected calcium improved to 7.3 mgs/dl (7.7–10.4) and then 9.3 mgs/dl, 7 and 15 days following admission respectively. Echocardiogram revealed good biventricular function on discharged. We understand that the mother had severe vitamin D deficiency and hypocalcemia during pregnancy and on low dose calcium and vitamin D tablets. On discharge mother was given six lakh units of ergocalciferol intramuscularly and started on calcium tablets. On further review calcium remained normal, the dosage of both calcium and calcitriol were reduced and later stopped. Three months later calcium and PTH remained normal.

Conclusion

The prevalence of fetal hypocalcemia secondary to maternal vitamin D deficiency is not well documented. Screening for this condition early in pregnancy should be included as part of routine investigation. This policy might reduce fetal morbidity and mortality.

DOI: 10.1530/endoabs.34.P256

P257

Contribution of hepatic liver X receptor to the adaptations in maternal lipid metabolism during pregnancy

Vanya Nikolova[1], Shadi Abu-Hayyeh[1], Georgia Papacleovoulou[1], Malcolm Parker[2] & Catherine Williamson[1]
[1]King's College London, London, UK; [2]Imperial College London, London, UK.

Introduction

Normal pregnancy is characterised by dyslipidaemia which progresses with gestational age as a consequence of the growing energy demands of the mother and developing fetus. Specifically, there is an increase in the maternal plasma cholesterol, triglyceride and phospholipid concentrations.

Liver X receptor (Lxr) is a nuclear receptor which promotes cholesterol clearance and *de novo* lipogenesis.

Hypothesis

We hypothesise that gestational dyslipidaemia is a result of pregnancy signals which modulate the activity of Lxr, thus altering the expression of Lxr target genes involved in the regulation of lipid homeostasis.

Methods and results

RNA studies in normal-chow-fed C57BL6 WT mice revealed that on day 7 post coitum (pcm) there was increased expression of Lxr target genes (e.g Scd-1, Cyp7a1, Abcg8), while on days 10, 14 and 18 pcm their expression was down-regulated. No gestational alterations in the mRNA and protein levels of Lxrα were observed. Attempting to reverse the gestational adaptations in lipid metabolism, we challenged pregnant mice with the Lxr agonist T0901317 through diet. Our results confirmed that the expression of the Lxr targets in pregnant T0901317-fed mice was significantly higher than in matched pregnant normal-chow-fed females. The gradual decrease in the expression of Lxr targets was maintained in the T0901317-fed pregnant mice but the magnitude of reduction was lower than that in normal-chow-fed mice. Biochemical profiling demonstrated that Lxr induction in pregnancy prevented hepatic and serum lipid maternal adaptations that were observed in normal pregnancy.

Conclusion

Our data reveal a gradual down-regulation of Lxr targets involved in hepatic lipogenesis and cholesterol transport following mouse placenta formation. Activation of Lxr not only blunted the reduction of these genes but also reversed the changes in hepatic and lipid profiles observed during normal murine pregnancy. Our results suggest that the adaptations in lipid metabolism during pregnancy are associated with a reduction in Lxr activity.

DOI: 10.1530/endoabs.34.P257

P258

High serum amylase a contraindication to GLP1 analogue treatment?

Karolina Stepien[1], Anne-Marie Kelly[1], Graham Horsman[1] & Naveed Younis[2]
[1]Clinical Biochemistry Department, University Hospital of South Manchester, Manchester, UK; [2]Diabetes and Endocrinology Department, University Hospital of South Manchester, Manchester, UK.

Case
A 41 year-old-Indian woman with type 2 diabetes treated with liraglutide was admitted to the Emergency Department with abdominal pain, nausea, vomiting and bloated abdomen. She was found to have raised amylase at 706 U/l. Abdominal ultrasound and CT scan did not reveal any abnormalities in the pancreas or billary tract and the patient was discharged.
She had diabetes for 9 years duration treated with metformin, pioglitazone and liraglutide. Over 2 years the dose of GLP-1 analogue was gradually increased from 0.6 to 1.8 mg at the presentation. Previously, her serum amylase showed variable results of 1481 U/l in her first pregnancy and between 62 and 249 U/l during her second pregnancy and this was attributed to parotitis.
In view of the raised amylase result and the likelihood of pancreatitis that may be associated with GLP-1 analogue treatment, the medication was stopped and she was regularly followed- up in the Diabetes Clinic. Her amylase remained raised despite the patient having no symptoms suggestive of pancreatitis.
A number of biochemical investigations can help determine whether or not a raised serum amylase may be due to pancreatitis. In this patient a normal serum lipase excluded a pancreatic origin, and a normal urine amylase fractional excretion (0.5%) added to the suspicion that the raised amylase in the absence of symptoms was due to macroamylasaemia. This was confirmed initially by PEG precipitation in the local laboratory and subsequently gel filtration chromatography in a specialised centre.
Conclusion
This is a case of chronically raised amylase but no clinical picture of pancreatitis. Macroamylasaemia is a rare benign condition that does not require treatment. The human GLP-1 analogues have been previously implicated as a risk for pancreatitis in humans. The decision to withhold GLP-1 analogues in this patient can be re-visited in the light of this diagnosis.

DOI: 10.1530/endoabs.34.P258

P259

Decrums disease: an unusual case of bilateral adrenal lipomas

Anne Marie Hannon, Lisa Owens & Domhnall O'Halloran
Cork University Hospital, Cork, Ireland.

Decrum's disease or adiposis dolorasa is a rare progressive syndrome of unknown aetiology. It is characterised by painful fatty deposits, general obesity, weakness and unexplained emotional disturbance.[1]
We describe a case of a 55-year-old man who presented with pneumonia. On examination he was noted to be overweight and have multiple skin nodules. His chest X-ray was abnormal; he underwent further workup with a CT thorax/abdomen/pelvis. This revealed no lung abnormality but demonstrated bilateral adrenal lesions. Biochemical testing revealed these were non-functioning lesions. He underwent an MRI of the adrenals, which showed changes consistent with bilateral lipomas (2.6 cm on left, <2 cm on right). He denied any previous past medical history or any family history of note.
One of his skin lesions was biopsied; this showed mature adipose tissue with focal loose stroma, containing mast cells, consistent with a lipoma.
The WHO classifies Decrum's disease as rare. It is typically associated with overweight, postmenopausal women. It may be inherited in an autosomal dominant manner, however most reported cases are sporadic.[2] It has multiple associated conditions including pickwickian syndrome, dry eyes and mouth; sleep disturbance, irritable bowel and carpel tunnel syndrome.
On review of the literature, we were unable to identify any case reports of Decrum's disease and adrenal lipomas, however typically it is believed they can occur anywhere in the body except the head and neck.[3] This case illustrates an unusual cause of adrenal lipomas.
References
1. Brodovsky S, Westreich M, Leibowitz A & Schwartz Y. Adiposis dolorosa (Dercum's disease): 10-year follow-up *Ann Plast Surg*. 1994 **33** (6) 664–8.
2. Lynch HT & Harlan WL. Hereditary factors in adiposis dolorosa (Dercum's Disease). *Am J Hum Genet*. 1963 **15** (2) 184–90.
3. Hansson E, Svensson H & Brorson H. Review of Dercum's disease and proposal of diagnostic criteria, diagnostic methods, classification and management. *Orphanet Journal of Rare Diseases*. 2012 **7** 23.

DOI: 10.1530/endoabs.34.P259

P260

Longitudinal changes in glucocorticoid metabolism predict the development of metabolic phenotype

Rachel Crowley[1], Beverly Hughes[1], Joanna Gray[2], Theresa McCarthy[2], Susan Hughes[1], Cedric Shackleton[3], Nicola Crabtree[2], Peter Nightingale[2], Paul Stewart[1] & Jeremy Tomlinson[1]
[1]Centre for Endocrinology, Diabetes and Metabolism, University of Birmingham, Birmingham, UK; [2]NIHR / Wellcome Trust Clinical Research Facility, Queen Elizabeth Hospital, Birmingham, UK; [3]Children's Hospital, Oakland's Research Institute, Oakland, California , USA.

Dysregulation of the enzymes that control local tissue steroid metabolism has been implicated in the pathogenesis of obesity and insulin resistant states, however longitudinal changes in glucocorticoid metabolism over time have not been investigated. This study was designed to evaluate the role of pre-receptor glucocorticoid metabolism in the development of insulin resistance and obesity. 24 h urinary glucocorticoid and mineralocorticoid metabolites were measured by gas chromatography/mass spectrometry in obese and overweight patients serially over 5 years and analysed in the context of dysglycaemia and hypertension at final visit, in order to identify potential biomarkers for the future development of metabolic disease.
Higher 5α-reductase activity, but not 11β-hydroxysteroid dehydrogenase type 1 activity, at study baseline was predictive of later development of increased fasting insulin (11.4 compared to 7.4 mU/l in subjects with lower 5αR activity, $P<0.05$), insulin response to oral glucose tolerance test (area under the curve for insulin 176.7 compared to 89. mU/l.h, $P<0.01$) and insulin resistance (HOMA2-IR 1.3 compared to 0.8, $P<0.01$) in obese and overweight subjects. Higher total glucocorticoid production was associated with abnormal glucose tolerance and increased BMI. Increased activation of the mineralocorticoid receptor during the study was indicated by increasing systolic blood pressure (equivalent to $\sim$ 1 mmHg/year) and plasma sodium levels over the 5 years of the study; mineralocorticoid activity was associated with increased aldosterone and decreased 11β-hydroxysteroid dehydrogenase type 2 activity.
This is the first longitudinal analysis of corticosteroid secretion and metabolism and its relationship with metabolic phenotype. Increased 5α-reductase activity and glucocorticoid secretion rate over time are linked to the development of metabolic disease, and may represent targets for therapeutic intervention.

DOI: 10.1530/endoabs.34.P260

P261

Abstract Withdrawn.

DOI: 10.1530/endoabs.34.P261

P262

Elevated cord leptin from low B12 mothers predicts birth weight

Adaikala Antonysunil[1], Manu Vatish[1,2], Alexander Lawson[3], Catherine Wood[4], Kavitha Sivakumar[1], Craig Webster[3], Neil Anderson[4], Philip McTernan[1], Gyanendra Tripathi[1] & Ponnusamy Saravanan[1,4]
[1]University of Warwick, Warwick, UK; [2]University of Oxford, Oxford, UK; [3]Heartlands Hospital, Birmingham, UK; [4]George Eliot Hospital, Nuneaton, UK.

Background
Vitamin B12 (B12) insufficiency is common in pregnancy and independently predicts insulin resistance (IR) in the offspring. B12 is an important key nutrient for epigenetic programming through regulating DNA methylation. Such B12 DNA methylation may influence leptin, a strong candidate for methylation, which could impact both insulin resistance (IR) and associated neonatal metabolic risk. Therefore, we hypothesize that leptin can be programmed by maternal B12 which could influence metabolic risk in the offspring. To test this hypothesis, we investigated whether i) maternal B12 is associated with leptin in cord blood and ii) evaluated their association with birth weight.
Methods
Paired maternal venous and cord blood samples ($n=91$) were collected at the time of elective caesarean section. Serum vitamin-B12 was determined by electro-chemiluminescent immunoassay. Leptin levels were measured by ELISA.

Results
B12 insufficiency (<150 pmol/l) was common (mothers-40%; and neonates-29%). Maternal B12 was inversely associated with neonatal leptin ($r=-0.304$; $P=0.005$). In regression analysis, adjusted for all likely confounders, maternal B12 independently predicted neonatal leptin ($\beta=-0.647$; $P=0.005$; $R^2=12.8\%$). There was no correlation between maternal and neonatal leptin levels. Cord leptin from mothers with low B12 correlated with birth weight ($r=0.366$; $P=0.036$). Regression analysis adjusted for maternal leptin and insulin showed that cord leptin from mothers with low B12 independently predicted birth weight ($\beta=0.024$; $P=0.049$; $R^2=14.5\%$).
Conclusion
Our study highlights that maternal B12 insufficiency predicts elevated leptin in cord blood and is associated with higher birth weight. Since cord leptin is derived from neonatal adipose tissue and not mother, these findings suggest that maternal B12 might program leptin levels *in-utero* either directly through the satiety centre or mediated via inducing IR and adiposity in the offspring. Delineating the mechanistic relationship between cord leptin and maternal B12 might provide crucial answers in understanding the molecular mechanisms of adverse metabolic programming.

DOI: 10.1530/endoabs.34.P262

P263

Renalase a key regulator of brown adipose tissue activity

Manjunath Ramanjaneya[1], Bee Tan[1], Vanlata Patel[1], Jiamiao Hu[1], Hendrik Lehnert[1,2], Edward Hillhouse[3], Jing Chen[1] & Harpal Randeva[1]
[1]University of Warwick, Coventry, UK; [2]University of Lübeck, Lübeck, Germany; [3]Hamad Medical Corporation, Doha, Qatar.

Renalase is a recently discovered flavin adenine dinucleotide (FAD)-dependent oxidase, predominantly secreted into the circulation from the kidney and regulates catecholamine degradation and metabolism. Renalase gene is also present in heart muscle, skeletal muscle and liver cells in humans, and in mouse testicles. Four isoforms of renalase gene has been identified in humans (hRenalase1 to hRenalase4), however only hRenalase1 is detected in human blood suggesting that hRenalase two to four may have alternative function. Unlike the classical amine oxidases which are expressed intracellularly, renalase is present intracellularly and is also secreted into the circulation. Circulating renalase appears to mirror sympathetic tone, a brief increase in catecholamines results in significant up-regulation of renalase synthesis, secretion and activity. Catecholamines are principle activators of BAT thermogenesis and angiogenesis both in rodents and humans. Characterization studies in renalase-knockout mice shows that these mice have about 25% reduced body weight compared to control, increased circulating catecholamines, are hypertensive, and have cardiovascular defects. Renalase-knockout mice have activated sympathetic system and possibly have increased BAT activity as a result of elevated catecholamine's in the circulation.
Our findings in adipose tissue demonstrate that the renalase gene and protein is expressed in both white and brown adipose tissue depots. Expression of renalase increases in BAT of mice during non-shivering thermogenesis and in diet-induced obese mice. The expression of renalase gene, protein and secretion are significantly higher in differentiated mature brown and white adipocytes compared to preadipocytes. Stimulation of mature brown adipocytes with norepinephrine, the main catecholamine released during cold induced non-shivering thermogenesis *in vivo*, results in significant increase in renalase expression suggesting the importance of renalase in non-shivering thermogenesis. In white adipocytes, renalase expression is up-regulated in response to treatment with obesity linked adipokines, specifically, leptin and insulin. We report renalase as a novel adipokine expressed and secreted from both BAT and WAT.
From our preliminary findings and observations in renalase knockout mice having increased sympathetic activity and circulating catecholamines, we hypothesise 'renalase regulates BAT development, thermogenesis and metabolism'. Therefore further studies are needed in renalase knockout mice to understand the role of renalase in adipose tissue metabolism which could provide us clues on its role in obesity and insulin resistance. Understanding the mechanisms of renalase action may provide translational data that underpins future treatment in obesity associated cardio-metabolic complications.

DOI: 10.1530/endoabs.34.P263

P264

Bariatric surgery for hypothalamic obesity: case series report

Ye Kyaw, Robert Mayo & Gul Bano
St George's Hospital, London, UK.

Introduction
Hypothalamic obesity can be associated with multiple co-morbidie and is difficult to treatment. There is little evidence regarding the effectiveness of currently available treatment for weight loss. We looked at the role of bariatric surgery on weight loss in patients with hypothalamic obesity at St George's Hospital, London.
Methods
Six patients with hypothalamic obesity had bariatric surgery. All patients had associated endocrine deficit secondary to pituitary disease. Patients were evaluated at 12–18 months postoperatively.
Findings
The average age of the patients was 42 years. Patients had Roux-en-Y gastric bypass and gastric sleeve. Greatest weight loss was achieved with the Roux-en-Y gastric bypass. Patients with Roux-en-Y gastric bypass. lost an average of 29.3% of their original bodyweight. Patients with gastric sleeve procedure lost an average of 25.1%.
Weight loss ranged from 19.8 to 66.9 kg. Despite, all comorbidities, all the patients achieved significant weight loss.
Conclusion
Bariatric surgery can be offered as an effective treatment for hypothalamic obesity. It produces substantial weight loss. The Roux-en-Y gastric bypass appears to achieve better weight loss when compared to the gastric sleeve. A long-term study to see the effectiveness of bariatric surgery and its effects on hormonal replacement is warranted.

DOI: 10.1530/endoabs.34.P264

P265

Depletion of glucose-6-phosphate transporter impacts SR calcium homeostasis in muscle

Craig Doig, Agnieszka Zielinska, Rachel Fletcher, Emma McCabe & Gareth Lavery
University of Birmingham, West Midlands, UK.

Glycogen storage disease type 1b is a metabolic disorder resulting in an inability to shuttle glucose-6-phosphate across the sarcoplasmic/endoplasmic reticulum (SR/ER) lumen. Mutation of the SoLute Carrier 37a4 (slc37a4) or glucose-6-phosphate transporter (G6PT) gene responsible for the distribution of G-6-P across this membrane leads to, hypoglycemia, hepatic glycogen accumulation, hyperlipidemia, resulting in life-limiting outcomes including growth retardation and neutropenia. Slc37a4 is highly expressed in metabolic tissues such as liver, kidneys and neutrophils. Moreover, expression is also found in muscle, a tissue that does not express the G-6-Pase system. Thus, the role of G6PT action in muscle is incompletely understood and how it contributes to metabolic implications of G6PT deficiency. G6PT $-/-$ mice are not viable, so we have begun to assess G6PT $+/-$ mice as model of G6PT deficiency to begin assessing its roles within muscle.
G6PT $+/-$ mice have $\sim$ a 50% reduction in protein levels. G6P in the SR is metabolised by H6PDH, generating NADPH for 11β-HSD1 and the generation of glucocorticoids. This 50% reduction did not affect 11β-HSD1 activity, and so is not rate-limiting for this pathway reaction. Using primary cultures of quadriceps muscle as a model we have identified a defect in intracellular calcium homeostasis. Using both colourmetric calcium detection assays and Fura-4 free-calcium binding assays we demonstrate greater accumulation of free calcium in G6PT $+/-$ muscle compared to WT. We also show that this defect in calcium is associated with increased expression of the SERCA2/ATPase pump responsible for flux of calcium into the SR lumen from the cytosol. These data suggest that the G6PT can influence calcium handling in muscle cells and that G6PT $+/-$ muscle has dysregulated calcium homeostasis. How G6PT influences muscle calcium is unknown. These studies highlight a novel aspect of calcium regulation in muscle that may have implications for patients with GSD1b.

DOI: 10.1530/endoabs.34.P265

P266

Breaking NAD^+: investigating NMRK2 as a regulator of muscle adaptation through NAD^+ salvage

Craig Doig, Agnieszka Zielinska, Rachel Fletcher, Emma McCabe, Andrew Philp & Gareth Lavery
Univeristy of Birmingham, West Midlands, UK.

Exercise has undisputed health benefits mediated through metabolic adaptation in skeletal muscle. In contrast, a sedentary lifestyle, leads to impaired metabolic function and negative health outcomes such as insulin resistance and sarcopenia. Whilst exercise improves skeletal muscle metabolism, how the process is co-ordinated at a cellular level remains unclear. Recently, NAD^+ has been suggested to play an important role in muscle adaptation, acting as a substrate for NAD^+-sensing sirtuin (SIRT) family of deacetylases linking NAD^+ signalling to adaptation. A newly identified source of NAD^+ is salvage of the dietary

vitamin nicotinamide riboside (NR) through the nicotinamide riboside kinase 2 (NMRK2) pathway. However, there is little known about the contribution of NMRK2 to NAD^+ salvage and maintenance of NAD^+ in skeletal muscle.
Recent studies investigating muscle in hexose-6-phosphate dehydrogenase (H6PDH) KO mice identified NMRK2 as a regulated stress response gene, being up-regulated (>50-fold in fast twitch muscle and greater than tenfold in slow twitch muscle), and associated with increased NAD^+ levels. NMRK2 expression increases during muscle differentiation and shows a dose-dependent increases in expression following overnight glucose restriction (shifting from 25 to 4 mM), again associated with increased NAD^+ levels. Conversely, myotubes transfected with NMRK2 siRNA had a significant reduction in cellular NAD^+(~30%). Knockdown of NMRK2 also had significant effects on the expression of mitochondrial NAD^+ dependent and NAD^+ associated enzymes methylenetetrahydrofolate dehydrogenase (folate biosynthesis), citrate synthase (TCA cycle) and mitochondrial cytochrome oxidaseC1 (fatty acid oxidation). Using microarrays we can show that NMRK2 is the most abundantly expressed gene amongst the few genes in muscle that regulate NAD^+ biosynthesis. Based on these ongoing studies, which are now focusing on sirtuin activity, we hypothesise that NMRK2 is a fundamental regulator of NAD^+ biosynthesis and is crucial to the ability of muscle to regulate metabolic adaptation and homeostasis.

DOI: 10.1530/endoabs.34.P266

P267

11β HSD1KO mice resist aged associated decline in markers of brown adipose tissue function

Emma McCabe, Craig Doig, Stuart Morgan, Dean Larner, Jeremy Tomlinson, Paul Stewart & Gareth Lavery
University of Birmingham, Birmingham, UK.

The primary function of brown adipose tissue (BAT) is to use lipids to generate heat through uncoupling of oxidative phosphorylation in mitochondria. Glucocorticoids (GC) have a negative effect upon BAT through inhibition of uncoupling protein 1 (UCP1) expression. Similarly, it has been reported that BAT levels decline with age and have been linked to age related accumulation of body fat, leading to the idea that improving BAT function during ageing could have a beneficial role in preventing weight gain due to its role in whole body energy expenditure. In this study we test the hypothesis that knock-out of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), which regenerates active GC from inactive precursors, will have beneficial effects on the function of BAT, and that this beneficial effect will be magnified during ageing.
WT and global 11β-HSD1KO (GKO) mice were aged to either 15 weeks (young) or 100 weeks (aged) and BAT adipose tissue collected, weighed and analysed. Firstly, aged WT mice showed dramatically elevated 11β-HSD1 mRNA and protein compared to young mice. BAT mass and markers of differentiation and function were largely unchanged in young GKO compared to WT mice. However, the loss of 11β-HSD1 up-regulation in aged GKO mice resulted in a significant increase in BAT mass. Though histologically indistinguishable, GKO mice also had significantly enhanced mRNA expression of UCP1, PGC1a, PPARg, cox8b, and cox7a1 and at the protein level of UCP1. Further to this, GKO mice had a significantly increased number of mitochondria and blotting for electron transport chain complexes 1–5 identified increased expression of the NADH dehydrogenase 1β subcomplex subunit 8, succinate dehydrogenase iron-sulfur subunit compared to WT. These data demonstrate that abrogation of age associated 11β-HSD1 up-regulation and depletion of 11β-HSD1 mediated GC reactivation has a beneficial effect on markers of BAT function, highlighting the importance of 11β-HSD1 during BAT ageing.

DOI: 10.1530/endoabs.34.P267

P268

H6PDH deficiency in muscle impacts amino acid metabolism

Agnieszka Zielinska[1], Craig Doig[1], Paul Stewart[2], Jerzy Adamski[3] & Gareth Lavery[1]
[1]University of Birmingham, Birmingham, UK; [2]University of Leeds, Leeds, UK; [3]German Research Center for Environmental Health, Neuherberg, Germany.

Hexose-6-phosphate dehydrogenase is an important factor in setting the redox status of the endo-/sarcoplasmic reticulum (ER/SR) lumen by generating the NADPH:$NADP^+$ ratio for 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) mediated glucocorticoid (GC) activation. H6PDH knockout mice (H6KO) clearly demonstrate the obligate nature of 11β-HSD1 for H6PDH, and display a vacuolating of type IIb fiber myopathy, elevated glycogen storage and type II to type I fibre type switching. They also display fasting hypoglycaemia, GC insensitivity and reduced skeletal muscle mass. To identify factors interacting with H6PDH and initiating myopathy, we have applied targeted metabolomics technology for quantification of 163 metabolites in quadriceps muscle from 8 weeks old H6KO and control mice ($n=10$). Of the 163 metabolites, 14 were amino acids of which Arg, Trp, Gly, Gln, and Pro were significantly elevated whereas Orn and xLeu significantly decreased. These changes reflected a diminished Orn:Arg ratio representing greatly impaired arginase activity. Additionally, we have screened the mRNA expression of most differentially regulated genes previously identified in H6KO myopathy-affected muscle by real-time PCR, and we have found a set of significantly deregulated genes involved in amino acids metabolism, with Gls, Gpt, Smox1, and Amd1 expression was decreased and Pycr1 and Aldh18a1 expression increased.Our preliminary data suggest that H6KO impacts amino acid metabolism that could contribute to aspects of the phenotype such as insulin sensitisation, such as elevated arginine and subsequent nitric oxide content, whereas changes in protein synthesis and amino acid accumulation might lead to a reduction in muscle mass and influence ER stress. These data are being analysed further using informatic approaches and may offer insight into homeostatic responses on amino acid metabolism during metabolic stress in skeletal muscle.

DOI: 10.1530/endoabs.34.P268

P269

Unfolded protein response in adipose tissue of obese diabetic women significantly improved 6 months post bariatric surgery, irrespective of malabsorptive or bypass operation type and correlates with plasma glucose concentration

Philip Voyias[1], Adaikala Antonysunil[1], Warunee Kumasaiyai[1], Ioannis Kyrou[1], Jana Vrbikova[2], Vojtech Hainer[2], Martin Fried[3], Petra Sramkova[3], Ponnusamy Saravanan[1], Sudhesh Kumar[1], Philip McTernan[1] & Gyanendra Tripathi[1]
[1]University of Warwick, Coventry, UK; [2]Institute of Endocrinology, Prague, Czech Republic; [3]OB Clinic, Prague, Czech Republic.

Background
In obesity, excess nutrients and an increased demand for protein synthesis contribute to unfolded proteins accumulating within the endoplasmic reticulum and consequent activation of unfolded protein response (UPR). UPR in adipose tissue (AT) is critical to the initiation and integration of inflammation and insulin signalling pathways in obese and type 2 diabetes mellitus (T2DM) patients. The aim of this study was to examine whether novel malabsorptive or bypass bariatric surgery in obese women with T2DM leads to reduction in UPR.
Methods
Abdominal subcutaneous (AbSc) AT was isolated from 30 Caucasian obese T2DM women aged 54.1±1.3 (mean±S.E.M.) years, BMI 41.21±1.0 kg/m^2, that had undergone bariatric surgery of malabsorptive; gastric band ($n=9$) or novel gastric plication ($n=13$), or bypass; biliopancreatic diversion ($n=8$) type. Biopsies and anthropometric data were collected at the time of surgery and 6 months post-surgery. UPR markers were measured by qRT-PCR and western blotting and correlation analysis was performed.
Results
Six months post-operation all subjects significantly reduced body weight ($P<0.001$) with mean excess BMI lost 33.4±2.4%. Anthropometric measurements were significantly improved; fat mass, HbA1c, glucose, insulin, HOMA-IR, and total cholesterol (all $P<0.001$). ATF6, IRE1α, XBP1s, ATF4, and CHOP10 mRNAs and ATF6, pIRE1α, XBP1s, Calnexin and Bip proteins were all significantly ($P<0.05$) reduced post-surgery irrespective of operation type. Correlations between UPR mRNAs were strengthened post-surgery for ATF4 and CHOP10 ($P=0.041$–$P<0.001$) and IRE1α and ATF6 ($P=0.853$–$P<0.001$). Post-surgery plasma glucose correlated significantly ($P=0.034$) with XBP1s mRNA.
Conclusion
This study highlights that bariatric surgery induced weight loss is coupled with improved glucose homeostasis and reduced UPR expression in AT. Furthermore post weight loss there are enhanced associations identified between UPR and XBP1 in AT and plasma glucose which may arise due to improved glucose homeostasis. This suggests UPR regulation in AT is linked to plasma glucose levels which aligns to metabolic health.

DOI: 10.1530/endoabs.34.P269

P270

Does TCF7L2 polymorphisms increase the risk of gestational diabetes mellitus in South Indian population?

Nihal Thomas, D M Mahesh, Aaron Chapla, Johan Paul, N Shwetha, Flory Christina & H S Asha
Christian Medical College, Vellore, India.

Introduction
Genetic predisposition with environmental factors plays a significant role in the development of type 2 diabetes mellitus. Among the diabetogenic genes, the transcription factor 7-like 2 (TCF7L2), a member of the Wnt signalling pathway remains the strongest genetic determinant of type 2 diabetes risk in humans. Our aim was to investigate whether TCF7L2 variants that have previously been associated with type2 diabetes would also confer risk for gestational diabetes mellitus in South India population.
Materials and methods
In 166 unrelated women (117 women with gestational diabetes mellitus and 49 non-diabetic control subjects) DNA extraction was done using Gentra Puregene Blood Method during the period January 2012–April 2013. The primers were validated by Sanger sequencing (3130 Genetic Analyzer). We genotyped three TCF7L2 polymorphisms (rs7903146, rs12255372, and rs4506565) using TaqMan allelic discrimination assay in our Molecular Laboratory.
Results and discussion
In a small South Indian population, we have found that the odds ratio (OR) of TCF7L2 polymorphisms rs4506565, rs7903146, and rs12255372 were (OR = 3.75 (95% CI 0.75–18.53), $P = 0.08$), (OR = 1.77 (95% CI 0.503–6.263), $P = 0.37$), and (OR = 1.40 (95% CI 0.24–8.11), $P = 0.70$) respectively, when compared with controls for the occurrence of gestational diabetes. The common variants of TCF7L2 (rs7903146,rs12255372, and rs4506565) have been shown to be associated with diabetes mellitus in European (Cauchi *et al*. 2007) and Asian population (Chandak *et al*. 2006, Alami *et al*. 2012) (OR = 1.3–1.9). The association of GDM with these TCF7L2 variants has varied in different populations, in Mexican Americans (OR = 2.49 (95% CI 1.17–5.31), $P = 0.018$), Arab women (OR = 2.370, (95% CI 1.010–5.563, $P = 0.047$), and Scandinavian women (OR = 1.49 (95% CI 1.28–1.75), $P = 0.049$).
Conclusion
The TCF7L2 polymorphism rs4506565 showed a trend towards association with gestational diabetes, when compared to the other two common polymorphisms in TCF7L2 (rs7903146 and rs12255372). However, to confirm our findings further studies need to be performed in a larger population.

DOI: 10.1530/endoabs.34.P270

P271

Evaluating β-hydroxybutyrate as indicator for early termination of 72 h fast for spontaneous hypoglycaemia

Octavian-Ionut Cozar[1], Sondra Gorick[1], Anupam Brahma[2] & Francesca Swords[1]
[1]Norfolk and Norwich University Hospital, Norwich, UK; [2]West Suffolk NHS Foundation Trust, West Suffolk, UK.

Background
The gold standard investigation for suspected spontaneous hypoglycaemia is the supervised 72 h fast. This aims to 'capture' a hypoglycaemic episode, to confirm Whipple's triad, and to measure simultaneous insulin and C-peptide levels. These should confirm or refute endogenous hyperinsulinaemia.
75% patients with confirmed insulinoma actually develop hypoglycaemia within 24 h of fasting. However, some confirmed cases require significantly longer. Conversely, in many individuals in whom clinical suspicion is low, it remains uncertain how long is long enough to rule out spontaneous hypoglycaemia. We propose that ketone (β-hydroxybutyrate (BOHB)) testing may significantly shorten the test required in many such cases.
Method
Following new guidance in 2009, we introduced BOHB testing to our 72 h supervised fast protocol in 2010. We present a retrospective study of case notes and lab records of BOHB results in all patients undergoing a fast at our institution since this time.
Results
43 patients underwent a fast. Three were excluded because ketone testing had not been performed, and one because they self-discharged prior to 72 h. 39 patients (male:female 1:3.8) were analysed. Two had positive fasts and were later confirmed to have insulinoma. Neither of these patients demonstrated a rise in ketones over the 2.7 mmol/l threshold (maximum recorded: 1.9 and 0.2 mmol/l). 74% of the remainder demonstrated a rise in blood ketones to over 2.7 mmol/l during the fast: median 50.5 h.
Conclusions
A rise in BOHB levels during the 72 h fast confirms that the patient has complied with the fast. However, we propose that a rise in BOHB > 2.7 mmol/l is an excellent surrogate marker for hypoinsulinaemia and hence could rule out pathological hyperinsulinaemia (or IGF mediated hypoglycaemia). We therefore propose that a rise in BOHB > 2.7 mmol/l indicates a 'negative' fast allowing this unpleasant and expensive investigation to be terminated early in 74% patients.

DOI: 10.1530/endoabs.34.P271

P272

Liver fibrosis is common in Alstrom syndrome and can be identified using non-invasive tests

Jonathan Hazlehurst[1], Matthew Armstrong[2], Jayne Hodgkiss[3], Rachel Crowley[1], Tarekegn Geberhiwot[3], Phillip Newsome[2] & Jeremy Tomlinson[1]
[1]Centre for Endocrinology, Diabetes and Metabolism, University of Birmingham, Birmingham, UK; [2]Liver Research and NIHR Biomedical Research Unit in Liver Disease, University of Birmingham, Birmnigham, UK; [3]Queen Elizabeth Hospital Birmingham, Birmingham, UK.

Alstrom syndrome is an autosomal recessive ciliopathy that is characterised by increased body mass index, type 2 diabetes, retinal dystrophy, sensorineural hearing loss, cardiac fibrosis, and chronic kidney disease. Non-alcoholic fatty liver disease (NAFLD) in Alstrom patients ranges from simple steatosis, steatohepatitis and ultimately to fibrosis and cirrhosis. Nafld fibrosis score (http://nalfdscore.com/), enhanced liver fibrosis (ELF)-panel blood tests and Fibroscans are non-invasive methods to identify patients with NAFLD fibrosis without liver biopsy.
22 patients underwent a hepatological assessment at the multidisciplinary Alstrom clinic at University Hospitals Birmingham (68% males and age 29.6 ± 2.6) including history, examination, biochemistry, liver ultrasound, ELF-panel (elevated liver fibrosis blood tests) and a Fibroscan. Patients were typically obese (BMI 30.3 ± 1.3) with a high incidence of type 2 diabetes (68%) and dyslipidaemia (41%). 17/22 had a fatty liver on ultrasound. All patients had a valid Fibroscan. 10/22 patients had a liver stiffness measurement suggestive of significant fibrosis (≥ 8 kPa) (BMI 32 ± 2.2 and age 30.4 ± 3.9) and were more likely to be diabetic ($P = 0.03$). 8/10 had an ELF-panel all of which were confirmatory of moderate-significant fibrosis. 9/10 had an NFS score calculated (six low, two indeterminate, and one high). Of the remaining 12 patients with a Fibroscan < 8 kPa ten had an ELF-panel (three severe, six moderate, and one non/mild). one patient with a positive Fibroscan and high ELF proceeded to biopsy and had significant fibrosis.
Imaging shows that NAFLD is prevalent in Alstrom syndrome and there is likely to be an under recognised burden of fibrosis which can be diagnosed using non invasive blood tests and Fibroscan. The NAFLD fibrosis score which is the most readily available assessment underestimates fibrosis in this cohort which may be a feature of the disease whereby fibrosis occurs at a younger age and at a lower BMI.

DOI: 10.1530/endoabs.34.P272

P273

Familial adult hyperinsulinism due to genetic glucokinase activation: implications for therapeutic use of glucokinase activators

B G Challis[1], J Harris[1], A Sleigh[2], S M Orme[3], N Seevaratnam[4], K Dhatariya[5], H L Simpson[1] & R K Semple[1]
[1]Wellcome Trust, MRC Institute of Metabolic Science, Addenbrooke's Hospital, University of Cambridge, Cambridge, UK; [2]Wolfson Brain Imaging Centre, Addenbrooke's Hospital, Cambridge, UK; [3]Department of Endocrinology, St James's University Hospital, Leeds, UK; [4]Queen's Medical Centre, Nottingham, UK; [5]Elsie Bertram Diabetes Centre, Norfolk and Norwich University Hospitals NHS Foundation Trust, Norwich, UK.

Glucokinase (GCK) serves as the blood glucose 'sensor' in pancreatic β-cells, being critically involved in transducing elevated blood glucose into increased insulin secretion. Inactivating GCK mutations cause a subtype of maturity onset diabetes of the young (MODY), whereas activating mutations are a rare cause of hyperinsulinaemic hypoglycaemia, usually presenting in infancy.
We now describe the case of a 60-year-old woman who first presented with symptomatic hypoglycaemia in her fifth decade. Despite biochemical evidence of hyperinsulinaemic hypoglycaemia, extensive investigation failed to identify an insulin-secreting tumour. An extended oral glucose tolerance test demonstrated fasting hypoglycaemia that was exacerbated following a glucose challenge, consistent with dysregulated glucose-stimulated insulin release. Mutational analysis of the human GCK gene revealed a heterozygous activating mutation,

p.Val389Leu, in the patient and four other family members. Of these, two were undergoing extensive investigation elsewhere for recurrent hypoglycaemia presenting in adulthood, whilst the other two adult relatives were asymptomatic despite profound hypoglycaemia.
GCK is also expressed in non-pancreatic tissues including liver and GI tract, where it is expressed in enteroendocrine L cells that secrete GLP-1. In the liver, GCK mediates glucose clearance and has been implicated in lipogenesis and the pathogenesis of non-alcoholic fatty liver disease. Indeed, concerns have been raised that small molecule glucokinase activators used in the treatment of type 2 diabetes may drive hepatic steatosis and dyslipidaemia. However three members of the family studied with the GCK p.Val389Leu activating mutation had normal lipid profiles, hepatic triglyceride commensurate with their degree of adiposity, and normal rates of *de novo* lipogenesis. Consistent with previous reports two subjects with the p.Val389Leu mutation did not have significantly different levels of GLP-1 compared with controls.
In conclusion, activating GCK mutations are a rare cause of hyperinsulinaemic hypoglycaemia in adults and provide unique opportunities to study concepts relating to glucose sensing and therapeutic manipulation of GCK in man.

DOI: 10.1530/endoabs.34.P273

P274

Metabolic pathway analysis in choline and methionine deficient mice: new insights into the mechanism of steatosis and insulin resistance

Marcus Lyall[1], Jonathan Manning[1], Jennifer Hunter[2], Steve Anderton[1], Richard Meehan[2] & Amanda Drake[1]
[1]Queen Margaret Research Institute, Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK; [2]MRC Human Genetics Unit, Western General Hospital, University of Edinburgh, Edinburgh, UK.

Introduction
In rodents, dietary choline deficiency (CDD) results in macrovesicular hepatic steatosis and insulin sensitisation whereas methionine and choline deficient (MCDD) diets result in inflammatory fibrotic steatohepatitis with hepatic insulin resistance. The methyl donors choline and methionine are essential components of one-carbon metabolism. Our hypothesis was that methyl donor deficiency in mice would affect the expression of key genes in pathways of hepatic lipid biosynthesis, lipid disposal and insulin signal transduction.
Methods
Male C57BL/6 mice ($n=10$/group) were maintained on CDD, MCDD or control diet for 4 weeks. Hepatic triglyceride content was quantified by colorimetric analysis and histology analysed by light microscopy. Liver transcript profile was examined using the Illumina Mouseref-6 platform ($n=4$/group) and expression changes validated using quantitative PCR.
Results
Hepatic triglyceride levels were increased in CDD and MCDD ($P<0.05$) compared to controls. Histologically, CDD mice exhibited hepatic steatosis with mild inflammatory infiltrate whereas MCDD mice demonstrated steatosis, inflammatory infiltrate and periportal fibrosis. Mediators of *de novo* lipogenesis were transcriptionally suppressed in CDD and MCDD (Srebf1, ACSL1, ChREBP, ACACA, FASn, and SCD1; $P<0.001$). Pathways of triglyceride synthesis, mitochondrial and peroxisomal β oxidation were largely unaffected. Expression of ER associated mediators of triglyceride hydrolysis and VLDL assembly (PNPLA3 and the carboxylesterase enzymes: CES1d, CES1f, CES1g, and CES3b) were strikingly suppressed in MCDD mice ($P<0.001$), with a subgroup (Ced1d and PNPLA3) also suppressed in CDD ($P<0.05$). Two key mediators of insulin signal transduction (IRS2 and PDK1) were down-regulated in MCDD ($P<0.05$).
Conclusions
Hepatic steatosis in methyl donor deficiency may be promoted by down-regulation of CES enzymes required for TG hydrolysis and VLDL assembly. MCDD associated hepatic insulin resistance may be further exacerbated by IRS2 and pdk1 suppression.

DOI: 10.1530/endoabs.34.P274

P275

Evaluation of a crude extract administration of *Cnidoscolus chayamansa* in normal and diabetic Wistar rats

Francis Echeverría-Bobadilla[1], Andrea Ceballos-Arana[2], Daniel Sanchez-Ruz[2] & Elda Pacheco-Pantoja[2]
[1]Escuela de Nutrición, Universidad Anáhuac Mayab, Merida, Mexico; [2]Escuela de Medicina, Universidad Anáhuac Mayab, Merida, Mexico.

Diabetes is one of the main health problems in the world, thus it is well worthwhile to explore alternate treatments made from natural extracts. *Cnidoscolus chayamansa*, is an endemic plant from the Southeastern of Mexico known for its easy and cheap growing requirements. *C. chayamansa* contains dihydromyricetin a flavonoid that is believed exerts hypoglycemic effects. We obtained a hydro-ethanolic extract from the leaves of *C. chayamansa* (EEC), optimized and standardized through fractionation. We administered a concentrated extract of ECC to diabetic rats and a diluted infusion made with the EEC to normal rats.
Diabetes was induced with a single intraperitoneal streptozotocin dose (60 mg/kg). We compared a standard oral treatment of metformin (MET) against the EEC. The diabetic animals were treated with EEC ($n=5$), metformin (MET) ($n=5$), or water (control) ($n=3$). The results indicated that after 4 weeks of treatment the blood glucose levels decreased about 50% in the diabetic group treated either with metformin or EEC showing no significant difference between EEC and MET. The data from the correlation analysis between glucose change and weight gain change showed no significant association in a non-diabetic group, whereas the MET and EEC diabetic groups retrieved significant association ($P=0.003$ and $P=0.032$ respectively). In this case, we can conclude that there is no difference between the actions of EEC and MET, nonetheless there were more blindness outcomes in the group with MET ($n=4$) than in EEC group ($n=1$). Another experiment using an infusion of only 2% ECC administered *ad libitum* and under high carbohydrate diet showed significant decreases in blood glucose ($P=0.029$) compared with control group, which were drinking water and the same diet.
These observations are enticing and prospective work will include the investigation on the gene expression related to glucose metabolism when the EEC is administered.

DOI: 10.1530/endoabs.34.P275

P276

A pathway to investigate and manage patients complaining of symptoms suggestive of hypoglycaemia post Roux-en-Y gastric bypass surgery

George Tharakan, Harvinder Chahal, Alex Miras, Preeshila Behary, Sanjay Purkayastha, Sherif Hakky, Jo Boyle, Candace Bovril-Taylor, Asta Julnaite, Sarah Allen, Jonathan Cousins, Krishna Moorthy, Ahmed Ahmed & Tricia Tan
Imperial Weight Centre, London, UK.

Bariatric surgery for obesity remains the most effective method to achieve weight loss and improvements in mortality. However, as the number of procedures increases to match the rising burden of obesity, unusual metabolic complications are now coming to the fore. One example of this is postprandial hypoglycaemia (PPH). The incidence of PPH has been reported as $<1\%$ although this problem is underreported. A correct diagnosis is essential as the condition can be extremely disabling.
Intervention
At the Imperial Weight Centre, we have established a pathway to investigate patients who complain of hypoglycaemic symptoms. The purpose of these tests is to i) confirm the presence of hypoglycaemia; ii) determine whether this is postprandial or fasting; and iii) guide management. All patients undergo continuous glucose monitoring (CGMS) for 5 days, attend our investigation unit for a mixed meal tolerance test (MMT), followed by a prolonged oral glucose tolerance test (OGTT). If fasting hypoglycaemia is noted on CGMS, a 72 h fast is also performed.
Results
To date, 16 patients have undergone this standardised diagnostic algorithm. The results show that most patients do not develop hypoglycaemia after MTT, but do so after OGTT. None of the patients that had fasting hypoglycaemia on CGMS had a formally proven hypoglycaemia during a 72 h fast.
Impact
This standardised pathway avoids further unnecessary and invasive investigations for pancreatic insulinomas. If the metabolic tests are completely normal, alternative aetiologies should be sought.
Conclusions
Successful management is often accomplished by avoidance of high glycaemic index foods and a small amount of weight increase, to increase insulin resistance. In more resistant cases therapies may include acarbose, diazoxide and octreotide. These measures have avoided the need for invasive surgical interventions (e.g. gastric bypass reversal or pancreatectomies). PPH remains an under-recognised problem and effective management should be delivered through a multidisciplinary clinic.

DOI: 10.1530/endoabs.34.P276

P277

Proliferative retinopathy in pregnancy after bariatric surgery

Helen Dobson, Chitra Raj & Alia Munir
Pinderfields General Hospital, Wakefield, UK.

Worsening of retinopathy after bariatric surgery has been reported[1] and there has been a recent small cohort stating the same[2], however to our knowledge this is the first report of occurrence in pregnancy post bariatric surgery with a previous diagnosis of type 2 diabetes mellitus.

A 33-year-old female presented to the joint antenatal clinic at 28/40 gestation for a GTT.

She had undergone a Roux en y gastric bypass (01/2012) 11 months prior to this pregnancy so GTT was avoided to prevent dumping. BM monitoring was commenced. The referring midwife at booking stated she was told that her diabetes had 'gone away'. She had a history of type 2 diabetes mellitus since 2005, retinopathy and hypertension. Her BMI was 55 with a weight of 145 kg pre-surgery (she had a 10 kg weight loss with Liraglutide). Post-surgery her weight was 102 kg giving her a booking BMI of 36. Diabetic medications were discontinued. BMs were out of pregnancy targets at 28 weeks and metformin commenced. Given her past history urgent retinal examination was requested, revealing proliferative retinopathy and she underwent urgent pan-retinal laser. Particular attention was given to her micronutrients requirements. Her elective section was uneventful.

We know from the Swedish NHS birth register study that women are at an increased risk of pre-term and small for gestational age births post-surgery[3]. In addition a review of the key results of the Swedish Obese Subjects Trial in which diabetes prevention and remission were secondary end points showed after 2 years, 72% of patients with T2DM at baseline were in remission in the post-surgical group, however 50% relapsed after 10 years[4]. This case highlights the need to ensure appropriate education regarding the metabolic and fertility sequelae after surgery. Currently there is no UK guidance surrounding GDM screening post bariatric surgery, these may need to be patient and procedure specific.

References
1. Silva RA *et al. Ophth Surg Lasers Imag Retina* 2013.
2. Thomas RL. *ADA Poster* 2013.
3. Roos N *et al. BMJ* 2013 (Nov).
4. Sjostrom L. *J Int Med* 2013.

DOI: 10.1530/endoabs.34.P277

P278

Therapeutic durability of the GLP1-based therapy Liraglutide in patients with type 2 diabetes mellitus

Daniel Border[1,2], Wendy Clayton[1], Puja Thadani[1], Joseph Reed[3], Harpal Randeva[1,2] & Thomas Barber[1,2]
[1]University Hospitals Coventry and Warwickshire (UHCW), Coventry, UK; [2]Warwick Medical School (WMS), University of Warwick, Coventry, UK; [3]No Institution, Southampton, UK.

Introduction

Therapeutic durability of GLP1-based therapies in patients with type 2 diabetes mellitus (T2D) is incompletely understood. Our aim was to explore the therapeutic effects of GLP1 based therapies in patients with T2D.

Methods

This was a retrospective study on patients with T2D ($n=55$) who attend a specialist clinic at UHCW, Coventry, and who had been treated with the GLP1 based therapy, Liraglutide, for at least 6 months. A successful response to Liraglutide was defined as per NICE criteria, as a reduction in HbA1c by at least 1% and a reduction in body weight by at least 3%.

Results

Of the 55 patients included, 4 were excluded due to insufficient data. Of the remaining 51 patients, 23 (45%) responded successfully to Liraglutide therapy following 6 months of treatment. Of the 28 patients who failed to respond, the vast majority ($n=23$) had a satisfactory drop in *either* HbA1c or weight. Of those patients ($n=23$) who responded successfully to Liraglutide therapy, 13 (57%) remain on liraglutide therapy. Of these 13 patients, nine had an increase of HbA1c (mean increase 16.9% from 6-month value) following 20 months of therapy. Of the remaining four patients, one had a further increase in HbA1c (by 10% from the 6-month value) at 30 months of treatment.

Conclusions

Most patients with T2D appear to respond to initiation of Liraglutide therapy within the first 6 months of therapy, with just below half satisfying NICE criteria for successful therapy. After 6 months of therapy, there appears to be variable glycaemic response. Underlying progressive β-cell dysfunction is likely to be contributory, although there is a clear need to identify predictive baseline markers for successful response to initiation of GLP1-based therapies. Our data highlight the importance of close follow-up of patients on GLP1 based therapies.

DOI: 10.1530/endoabs.34.P278

Pituitary

P279

An unusual cause of pituitary apoplexy

Preeshila Behary, Gareth Townsey, Amrish Mehta & Karim Meeran
Imperial NHS Trust, London, UK.

Hypopituitarism secondary to pituitary apoplexy is a rare but recognised complication following cardiac surgery but not cardiac arrest.

We present a case report of acute pituitary apoplexy following a cardiac arrest on a background of sepsis. A 65-year-old gentleman presented with 3-day history of abdominal pain and vomiting. Of note, he underwent an appendicectomy 2 months earlier. He was septic and investigations revealed bowel perforation with faecal peritonitis. He underwent a hemicolectomy within 24 h of admission, but unfortunately suffered a cardiac arrest intra-operatively. Two cycles of CPR were administered after which his rhythm switched from PEA to pulseless ventricular fibrillation. He was cardioverted successfully to sinus rhythm and admitted to ITU. His blood pressure remained low and he required inotropic support. His serum sodium fell to 130 mmol/l over the next couple of days post his cardiac arrest. Hypo-cortisolaemia was suspected and a short Synacthen test performed. This showed an inadequate response with baseline morning cortisol of 69 nmol/l and a peak cortisol of 286 nmol/l at 60 min. ACTH was undetectable. Pituitary profile suggested pan-hypopituitarism: TSH 0.18 mIU/l, free T_4 <5.2 pmol/l, free $_3$ <1.5 pmol/l, LH 1.0 IU/l (NR 2–12 IU/l), FSH 1.5 IU/l (NR 1.7–8.0 IU/l), testosterone <0.3 nmol/l, prolactin 75 mU/l, GH <0.05 μg/l, and IGF1 5.8 nmol/l (NR 6–36 nmol/l). Pituitary MRI findings were suggestive of an infarct within an existing right sided pituitary tumour. He has since been started on hydrocortisone, thyroxine and testosterone replacement therapy.

Given the clinical presentation, we felt that a period of systemic hypo-perfusion during the cardiac arrest led to ischaemia and infarction of the existing pituitary tumour causing pan-hypopituitarism.

The prevalence of pituitary tumours is estimated at 17% and therefore it is important to consider the possibility of pituitary apoplexy in patients post cardiac arrest especially in the presence of hyponatraemia and hypotension.

DOI: 10.1530/endoabs.34.P279

P280

Pituitary metastasis from CLL – an extremely rare case

Kirthika Jeyaraman & Rashmi Kaushal
West Middlesex Hospital, London, UK.

We report an 84-year-old lady with stage A chronic lymphatic leukaemia which has been stable since 2002. She presented to our A&E in January 2013 with 3 days history of worsening frontal headache. She did not report visual disturbance or weakness. Examination revealed right sided homonymous hemianopia with no other neurological deficit.

White cell count and lymphocytes were 53.9×10^9/l and 42.0×10^9/l respectively. CT head showed an enlarged pituitary gland measuring 1.7 cm with features suggesting recent hemorrhage. MRI confirmed above findings and revealed optic chiasmal compression. CSF analysis showed elevated protein, normal glucose and no organisms were seen. CSF cytology was not performed. Blood tests were consistent with hypogonadotrophic hypogonadism. Cortisol was 6429 nmol/l, reduced to 1724 nmol/l after 2 days. Prolactin was undetectable. TFT and IGF1 were normal.

She underwent transsphenoidal hypophysectomy. The pituitary gland and hematoma were evacuated successfully with no immediate complications. Histology showed necrotic and haemorrhagic adenoma. Pathological features were consistent with apoplexy. It was thought that the pituitary vessels were clogged by CLL cells which led to apoplexy. Immunocytochemistry showed features diagnostic of leukemic infiltrate and consistent with chronic lymphocytic leukaemia. She developed hormone deficiency and Diabetes insipidus post surgery and is currently on hydrocortisone, thyroxine and desmopressin. She has residual right sided homonymous hemianopia. Haematologists are continuing to monitor her as usual.

Tumour metastasis to the pituitary gland is unusual and pituitary metastasis from CLL is extremely rare. Only 1% of the pituitary surgeries are performed for pituitary metastasis. The prognosis depends on the course of the primary neoplasm. Only 7% of pituitary metastasis are reported to be symptomatic with Diabetes insipidus being the most frequent presenting symptom in 50% of cases. Their rarity as well as the lack of specific clinical and radiological features impede their differentiation from other more common sellar lesions.

DOI: 10.1530/endoabs.34.P280

P281

Painful third nerve palsy caused by primary pituitary lymphoma associated with apoplectic pituitary adenoma

Luke Pickup & Adrian Jennings
The Queen Elizabeth Hospital King's Lynn NHS Foundation Trust, Norfolk, UK.

A 74-year-old male presented with acute onset retro-orbital pain and nausea. He also complained of blurred vision and inability to open his left eye. There was no family history and the only past medical history was hypercholesterolemia treated with simvastatin. Clinical examination revealed a complete left ptosis, the left pupil was dilated with sluggish reaction to light and there was impaired adduction of the left eye. Visual fields and visual acuity were normal. Examination was otherwise normal. Investigations revealed Na 131 mmol/l, other electrolytes normal. Endocrine investigations revealed serum testosterone 5.3 nmol/l, prolactin 701 mU/l, LH 1.1 IU/l, FSH 4.4 IU/l, free T4 10.03 pmol/l TSH 1.03 mIU/l. Short synacthen test; baseline cortisol 129 nmol/l, 60 min cortisol 921 nmol/l. MRI of the pituitary revealed a mass arising within the pituitary fossa extending into the left cavernous sinus and impinging on the inferior surface of the optic chiasm consistent with a pituitary adenoma. There was no evidence of apoplexy. The initial diagnosis was a non-functioning pituitary macroadenoma. The patient underwent transphenoidal resection, operative findings were consistent with a pituitary adenoma. Subsequent histology showed a neoplasm composed of diffuse sheets of malignant lymphoid cells, consistent with a high grade B-cell lymphoma. In addition there was a fragment of adenoma strongly immunoreactive for FSH which was partly necrotic, indicating a gonadatroph adenoma with a degree of apoplexy. Examination and imaging did not reveal evidence of lymphoma elsewhere.
In this case the main tumour was a primary pituitary lymphoma (PPL). However, we suspect that apoplexy of the pituitary adenoma caused the acute headache and contributed to the third nerve palsy. PPL is a rare condition which may present with features consistent with a pituitary adenoma; headache, cranial nerve dysfunction, and endocrinopathy. The only definitive way to confirm the diagnosis of PPL is with histology.

DOI: 10.1530/endoabs.34.P281

P282

Lack of Fpr2/Fpr3 receptors alters the structure and function of pituitary corticotrophs

Maria Mahmood & Helen Christian
Oxford University, Oxfordshire, UK.

Introduction
The *N*-formyl peptide receptor (FPR) family of G-protein-coupled receptors, originally identified to recognise *N*-formylated bacterial peptides, has in more recent times been shown to bind annexin-1 (ANXA1). ANXA1 is a signalling molecule well demonstrated to mediate two key glucocorticoid effects in the anterior pituitary: inhibition of ACTH release from corticotrophs and regulation of cell proliferation. Whilst previous RT-PCR studies have detected Fpr2, Fpr3, Fpr-rs6 and Fpr-rs7 receptor mRNA in murine pituitary tissue, the specific member(s) of the FPR family involved in ANXA1 signalling are unclear. In this study, we investigated whether glucocorticoid inhibitory feedback is impaired in Fpr2/Fpr3 double knockout (DKO) mice.
Method
Anterior pituitary tissue from WT and Fpr2/Fpr3 DKO male mice was fixed and examined i) by electron microscopy to determine corticotroph size, granule morphology and rough endoplasmic reticulum (rER) expansion, and ii) by immunocytochemistry to determine corticotroph density. Plasma ACTH and corticosterone concentrations were determined by RIA.
Results
In Fpr2/Fpr3 DKO mice, corticotrophs exhibited a significant ($P<0.01$) increase in rER expansion and a significant ($P<0.05$) decrease in granule density (%) suggesting increased ACTH synthesis and secretion corresponding with elevated levels of plasma ACTH ($P<0.05$) and corticosterone ($P<0.05$). Fpr2/Fpr3 DKO mice exhibited a significant ($P<0.01$) increase in corticotroph cell density.
Conclusion
These data suggest that there is a loss of ANXA1-mediated glucocorticoid action in Fpr2/Fpr3 DKO mice, indicating that Fpr2 and/or Fpr3 may function as ANXA1 receptor(s) in the anterior pituitary.

DOI: 10.1530/endoabs.34.P282

P283

A deficit of dystrophin 71 leads to disorder in vasopressin and oxytocin production in the magnocellular neurons

Roza Benabdesselam[1,2], Latifa Dorbani-Mamine[2], Alvaro Rendon[3] & Hélène Hardin-Pouzet[4]
[1]Faculté des Sciences Biologiques et Agronomiques/UMMTO, Tizi-Ouzou, Algeria; [2]Equipe de Neurochimie/LBPO/Faculté des Sciences Biologiques/USTHB, Bab Ezzouar, Alger, Algeria; [3]INSERM UMR S 592 LPCMR, Institut de la Vision, Paris, France; [4]UPMC/INSERM UMRS 952, CNRS UMR 7224, PMSNC, Paris, France.

Vasopressin (VP) and oxytocin (OT), the antidiuretic and natriuretic hormone, respectively, maintain the osmotic homeostasis. These molecules are synthesized in the magnocellular neurons (MCNs) of the supraoptic nuclei (SON) in the hypothalamus, transported via the axons toward the neural lobe of hypophysis (NLH) and released into the blood stream.
Dp71 is the major form of dystrophins in the SON and NLH, where it was located in the glial-end-feet and endothelial cell. To clarify Dp71's involvement in SON and NLN response to osmotic stimulus, we examined the effect of Dp71 disruption on the VP and OT synthesis in SON and release from NLH before and after salt-loading (SL).
In Dp71-null mice, SL increased VP-mRNA and VP-peptide, although this increase was smaller than that observed in WT. It is associated with an increase in VP export, insofar as the VP content was reduced in the SON of SL-Dp71-null mice, and the same pattern was observed in the NLH.
OT-mRNA and OT-peptide were more intense in SON of Dp71-null than in SON of WT before SL. SL increased OT-mRNA and OT-peptide in SON of WT but had no effect on those of Dp71-null. In the NLH, before SL, OT was lower in Dp71-null than in wild-type, and under SL, OT decreased in NLH of WT but not in that of Dp71-null.
To return to the pathology, recent studies described symptomatic nephrolithiasis in Duchenne muscular dystrophy patients. One of the origins of stone disease is a reduction in urinary volume. This could be the consequence of a change in the capacity of the SON and NLN to react to osmotic changes resulting from the disruption of dystrophin.

Key Words: dp71, vasopressin, ocytocin, supraoptic nucleuc, neural lobe, osmoregulation

DOI: 10.1530/endoabs.34.P283

P284

The effect of pregnancy on hyperprolactinaemia: a 5-year retrospective observational study

Vani Shankaran & Emma Ward
Leeds Teaching Hospitals NHS Trust, Leeds, UK.

Introduction
It is well-known that prolactinomas sometimes resolve following pregnancy. We wanted to see how often this happens and pregnancy outcome.
Patients and methods
From mid 2008 to mid 2013, 66 patients with hyperprolactinemia were studied. 39 had definite microprolactinomas, four probable microprolactinomas, three macroprolactinomas, eight nontumoral hyperprolactinemia and 12 patients were diagnosed elsewhere so no scan results were available. 54 out of 66 patients (82%) were on DA therapy prior to pregnancy, which was discontinued within 6–8 weeks of gestation. The incidence of miscarriage, foetal malformations and premature delivery were analysed.
Results
There were 51 (77.3%) live births and two miscarriages (3%).One patient (44 years) had termination for Down's syndrome. One had premature delivery secondary to chorioamnionitis resulting in neonatal death. There was no clear perinatal data available in 11/66 (16.7%). There were no neonatal malformations noted in all live births. Only four out of 66 patients needed to restart on DAs after lactation. 93.9% didn't require any further treatment, assuming that the prolactin was normal in those women whose prolactin was not rechecked following breastfeeding. All three with macroprolactinomas had a significantly lower prolactin after lactation compared to their initial level. Only one patient had tumour enlargement during pregnancy which required treatment and even she didn't require DA therapy after lactation. Patients with microprolactinomas were often discharged to GP to have a prolactin level checked after lactation. However, only 57.6% of patients had it done.
Conclusions
Foetal exposure to DAs at conception did not appear to increase risk of miscarriage/malformations. Pregnancy was associated with normalisation of prolactin levels in 93.9% of patients, including all macroprolactinomas. As the

majority of patients did not have a prolactin checked after they had finished breast feeding more rigorous follow up arrangements are needed.

DOI: 10.1530/endoabs.34.P284

P285

Diabetes insipidus in Erdheim-Chester disease

Nishant Ranjan[1], V P Sumathi[2] & Zayd Merza[1]
[1]Barnsley Hospital NHS Foundation Trust, Barnsley, South Yorkshire, UK; [2]Royal Orthopaedic Hospital Foundation Trust, Birmingham, UK.

A 26-year-old man presented with a 3-month history of polydipsia, polyuria and nocturia. Physical examination was unremarkable. Random blood glucose was 5.1 mmol/l, sodium 147 mmol/l, serum osmolality 297 mOsm/kg, urine osmolality 81 mOsm/kg. A water deprivation test confirmed cranial diabetes insipidus. Magnetic resonance imaging (MRI) of his pituitary gland revealed nodular thickening of the pituitary stalk suggestive of inflammatory or neoplastic aetiology. He also complained of pain in his right ankle for which he had an X-ray which showed diffuse permeative abnormality with periosteal reaction. MRI scan of his legs and a bone isotope scan revealed involvement of several other parts of his skeleton. Computerised tomography scan of his chest, abdomen and pelvis did not show any visceral involvement or lymphadenopathy. A bone biopsy of his right femur revealed diffuse infiltration of the medullary space by foamy histiocytes in a background of fibrosis and chronic nonspecific inflammation. The immunostaining of the foamy cells was negative for S100 and positive for CD68. The findings confirmed a diagnosis of Erdheim-Chester disease. He was commenced on desmopressin and further testing revealed low testosterone and GH deficiency.
Erdheim-Chester disease is a rare non-Langerhans form of histiocytosis characterised by xanthomatous infiltration of tissue with foamy histocytes with positive immunohistochemical staining for CD68 and negative for CD1a and in 80% of cases negative for S100 protein. Bone pain is the most common symptom, mainly affecting the lower limbs. It predominantly involves the skeletal system but other organs are involved including the pituitary gland typically causing diabetes insipidus. The first line of treatment is interferon alpha with a 5-year survival of 68%.

DOI: 10.1530/endoabs.34.P285

P286

The gonadotroph natriuretic peptide system is sensitive to pulsatile GnRH stimulation: insights into CNP/GC-B signalling in gonadotroph function

Samantha Mirczuk[1], Alice Catterick[1], Andrew Lessey[1], Rebecca Perrett[2], Craig McArdle[2], Imelda McGonnell[1] & Robert Fowkes[1]
[1]CBS, Royal Veterinary College, London, UK; [2]School of Clinical Sciences, University of Bristol, Bristol, UK.

Gonadotrophs in rats, mice and humans, express an intact natriuretic peptide system, in which C-type natriuretic peptide (CNP) is the predominant member. Despite showing an interaction between CNP and GnRH at the level of cGMP and Ca^{2+} signalling, the role of CNP in gonadotroph biology is poorly understood. In this study, we utilise a novel multiplex qRT-PCR assay, examining simultaneous expression of natriuretic peptide genes along with genes for gonadotroph transcription factors and signalling proteins, in a single PCR (*Nppa, Nppb, Nppc, Npr1, Npr2, Npr3, Corin, Furin, Nr5a1, Nr0b1, cFos, Egr1, cJun, Mkp1, Mkp2, Actb, Gapdh, Rpl19*). Initial expression screening in αT3-1, LβT2 and mouse pituitaries confirmed CNP (*Nppc*) and GC-B (*Npr2*) to be the predominant peptide and receptors present. We then challenged αT3-1 and LβT2 cells for 4 h with 100 nM GnRH, either chronically, or as 5 min pulse/hour, before examining gene expression. Chronic GnRH significantly inhibited *Corin* expression in αT3-1 cells ($*P<0.05$), but enhanced *Npr3* expression in LβT2 cells ($P<0.01$). In contrast, pulsatile GnRH significantly increased *Nppc*, *Furin*, *Npr2* and *Npr3* expression in LβT2 cells ($*P<0.05$, $***P<0.001$). As expected, both chronic and pulsatile GnRH caused significant changes in transcription factor and signalling protein gene expression in both cell lines. To establish the effects of CNP signalling on gonadotroph gene expression, αT3-1 and LβT2 cells were treated with 100 nM CNP for 0, 4, 8 and 24 h prior to multiplex qRT-PCR analyses. In αT3-1 cells, CNP stimulated *cFos*, *cJun* and *Mkp2* expression, but inhibited *Nr5a1*. In contrast, CNP stimulated *cJun*, *Egr1*, *Nr5a1*, *Nr0b1* and *Mkp1* expression in LβT2 cells. Collectively, these data provide the first evidence that the gonadotroph natriuretic peptide system is sensitive to pulsatile GnRH, and has identified putative CNP-target genes.

DOI: 10.1530/endoabs.34.P286

P287

An evolution of clinical practice: the impact of changes in clinical management of non-functioning pituitary adenomas on long-term pituitary function and risk of recurrence

Michael O'Reilly[1], Harriet Pearce[1], Gabriella Bugg[1], Rosalind Mitchell[2], Andy Toogood[1], Neil Gittoes[1] & John Ayuk[1]
[1]Department of Endocrinology, Queen Elizabeth Hospital, Edgbaston, Birmingham, UK; [2]Department of Neurosurgery, Queen Elizabeth Hospital, Edgbaston, Birmingham, UK.

Management of newly-diagnosed non-functioning pituitary adenomas (NFPAs) has evolved over the last decade. Whilst surgical debulking remains the mainstay of treatment for patients presenting with compressive disease, the use of pituitary irradiation has declined, with greater emphasis on observation or further surgical debulking. We aimed to compare outcomes of treatment for NFPAs at our institution since 2004 with older management strategies.
We reviewed records of 203 patients with NFPA referred for treatment to the Queen Elizabeth Hospital Birmingham between 1979 and 2012. Cases were subdivided into those presenting pre-2004 and post-2004. Treatment strategy at diagnosis was categorised as conservative, surgery or surgery plus radiotherapy (RTX). Tumour regrowth was diagnosed radiologically by a pituitary neuroradiologist. Pituitary function testing was performed preoperatively, 6 weeks postoperatively and annually thereafter.
203 patients were included (121 men) in the study. Mean age at diagnosis was 56.8 ± 14.5 years. 57 patients were treated pre-2004 and 146 post-2004 (mean follow-up 14.6 ± 6.5 and 6.7 ± 2.4 years, respectively). The incidence of intrasellar tumour at diagnosis in each group was 1/57 and 10/146, respectively, $P=0.002$. Cavernous sinus invasion was higher at diagnosis in the pre-2004 group (23/57 vs 37/146, $P=0.01$). The rate of postoperative surgery plus RTX was higher in the pre-2004 group (29.8 vs 2.7%, $P<0.0001$), as was the rate of long-term panhypopituitarism (53.4 vs 31.2%, $P=0.004$). Intra- or extrasellar tumour remnant was found postoperatively in 90.1% pre-2004 and 71.5% post-2004 ($P=0.03$). The rate of tumour regrowth was 39.2 and 17.8% pre-2004 and post-2004 respectively, $P=0.002$.
Lower rates of postoperative pituitary irradiation since 2004 have led to a significant reduction in long-term hypopituitarism in our cohort. More aggressive surgical debulking resulting in reduced postoperative tumour burden may have lowered rates of tumour regrowth in recent years at minimum expense to pituitary function. Long-term follow-up is required to validate these findings.

DOI: 10.1530/endoabs.34.P287

P288

A case of pancytopenia due to isolated ACTH deficiency successfully treated with hydrocortisone

Shiva Mongolu, Rajamani Manikandan, Jana Bujanova & Azraai Nasruddin
Southampton General Hospital, Southampton, UK.

Background
Pancytopenia due to hypopituitarism has been reported previously in the literature. Most of these case reports relate to hypopituitarism secondary to Sheehan's syndrome. We report a case of isolated ACTH deficiency causing pancytopenia which was successfully treated with hydrocortisone replacement.
Case history
A 71-year-old lady with a known history of treated hypothyroidism and learning disabilities was referred to Acute Medical Unit with urinary frequency, back pain and increased confusion. Urinary tract infection (UTI) was confirmed with positive urine culture for *E. coli*. Her diuretics were stopped, antibiotics started and despite IV fluid resuscitation, she remained hypotensive with hyponatraemia (Na 120) and acute kidney injury (Urea 6.8 Creat 169). Her synacthen test confirmed cortisol deficiency with 0 min cortisol 111 nmol/l, 30 min cortisol 291 nmol/l and 60 min 323 nmol/l. She was started on hydrocortisone replacement with good clinical improvement.
She was pancytopenic with WBC 2.1 Hb 98 g/l PLTS 53. Further investigations for her anaemia and pancytopenia failed to reveal any obvious cause. The reticulocyte count was normal and HIV, hepatitis serologies were negative. Direct antiglobulin test was positive, Urine Bence-Jones protein was negative. Bone marrow aspirate showed increased cellularity but no features of myelodysplasia.
Her ACTH was undetectable <5. Other anterior pituitary hormones showed prolactin 77 mu/l, IGF 26 μg/l, LH 34.2 IU/l, FSH 86.7 IU/l. TSH was 0.20 mU/l and free T4 20.1 pmol/l on 100 μg of thyroxine. The adrenal antibodies were negative and pituitary MRI was normal. A diagnosis of Isolated ACTH deficiency was made and she was discharged with outpatient Endocrine and Haematology follow-up. Within 2 months of hydrocortisone replacement, all her counts improved with Hb concentration 113 g/l, WBC 5.7s, PLTS 248 and remained stable.

Conclusion
To our knowledge this is the first reported case of isolated ACTH deficiency causing pancytopenia successfully treated with steroid replacement.

DOI: 10.1530/endoabs.34.P288

P289

Comparison of acute and subacute pituitary tumour apoplexy

Ioannis Dimitropoulos[1], Louis Pobereskin[2] & Daniel Flanagan[1]
[1]Plymouth Hospitals Nhs Trust, Derriford Hospital, Department of Diabetes and Endocrinology, Plymouth, Devon, UK; [2]Plymouth Hospitals Nhs Trust, Derriford Hospital, Department of Neurosurgery, Plymouth, Devon, UK.

Introduction
Pituitary apoplexy (PA) remains a rare endocrine diagnosis. Recent UK guidelines have emphasised the lack of evidence in the management of this condition. We present our experience of 23 current cases.
In classical acute PA, headache is the commonest presenting symptom. We found that a significant proportion (36%) of patients presented with subacute pituitary tumour apoplexy (SPA) – a term used to describe intra-adenomatous pituitary haemorrhage associated with clinical symptoms atypical of acute PA. These symptoms typically last longer than 24 h. In most previous case series, the incidence of SPA was found to be significantly lower than acute PA.
Presentation
Clinical suspicion of PA was high in 65% of cases: acute onset, severe headache in 65% of patients, and ocular palsy in 30% of cases with visual field defects in 13% of the patients. Over a third of patients presented with non-specific symptoms of fatigue, dizziness, nausea with -no or mild new headache, suggesting a diagnosis of SPA.
Management
The majority (83%) of cases were managed conservatively. Four (17%) of the patients with acute PA had severe neuro-ophthalmic signs and/or visual field defects and required and received urgent pituitary surgery with excellent post-operative outcome. None of the above cases required pituitary radiotherapy.
Subacute pituitary apoplexy subgroup
The group of patients with presumed SPA was on average younger than the acute PA subgroup. The requirement for long-term endocrinological hormone replacement in SPA is lower than in acute PA.
Conclusion
Although most cases presented with classical PA, a significant part presented with SPA. Clinicians need to be aware that SPA can present with non-specific symptoms.
Stable patients with PA or SPA without neurological or neuro-ophthalmic signs can be considered for a conservative management approach with careful monitoring in the context of a multi-disciplinary team which in our experience provides satisfactory outcomes.

DOI: 10.1530/endoabs.34.P289

P290

Newer dopaminergic agents cause minimal endocrine effects in subjects with idiopathic Parkinson's disease

J Daniel[1], J Govindan[2], C Kamath[2], C De souza[1], M A Adlan[2] & L D Premawardhana[2,3]
[1]Section of Car of The Elderly Medicine, Caerphilly miners' [2]Endocrinology and Diabetes Section, Caerphilly Miners and YYF hospitals, Caerphilly, UK; [3]Endocrinolgy and Diabetes, University Hospital of Wales, Cardiff, UK.

Objective
We studied the prevalence of endocrine dysfunction in subjects with idiopathic Parkinson's disease (IPD) on newer dopaminergic agents (DA). DA are used in endocrine hypersecretory states in small doses and we hypothesized that endocrine dysfunction was likely in IPD where DA was used in comparatively much higher dosage.
Patients and methods
25 subjects with IPD on DA for were recruited to this cross-sectional study. We measured IGF1, prolactin, LH, FSH, thyroid function, oestradiol or testosterone and cortisol following a short synacthen test.
Results
We studied 18 males and seven females (median age 72 years) whose median time from diagnosis, and duration of treatment was 27 months (interquartile range 17–45 and13–39 months respectively). i) Endocrine tests were normal in 19 subjects at recruitment. Minor abnormalities reverted to normal on repeat testing in three of six with initial abnormalities; two had persistent abnormalities; and the third subject could not be further investigated. Therefore 22 of 24 (92%) with IPD on DA therapy had normal endocrine profiles. ii) The cortisol response to ACTH was normal in 24 of 25 subjects (96%). iii) Eleven subjects (44%) had isolated PRL suppression. There were no differences between the suppressed PRL and 'normal' PRL groups. However, a higher number of them were on non-ergoline derived DA (83 vs 31%; $P < 0.05$).
Conclusions
We have demonstrated that newer non ergoline DA therapy caused only minimal endocrine perturbations in IPD subjects. The cortisol response to ACTH was normal in almost all but a significant minority had suppressed prolactin levels.

DOI: 10.1530/endoabs.34.P290

P291

Chronic glucocorticoid exposure inhibits expression of the *pomc* activator, *tpit,* by inducing *de-novo* DNA methylation

Georgia Bakirtzi & John Newell-Price
The University of Sheffield, Sheffield, UK.

Introduction
The HPA axis is essential for mammalian life. Glucocorticoids are commonly administered and long-term HPA axis suppression is a major clinical problem. Previous experiments in our lab have shown that long-term treatment with glucocorticoids cause silencing of *tpit*, a pituitary-specific master regulator of the key player in HPA axis regulation – propiomelanocortin (*pomc*). Even after withdrawal of treatment *tpit* expression is silenced.
Hypothesis
Chronic glucocorticoid exposure causes *de novo* DNA methylation of *tpit* inhibiting its expression and so influencing HPA axis regulation even after withdrawal of long-term glucocorticoid treatment.
Methods
Methylation patterns of *tpit* were studied by bisulphite sequencing in murine *POMC*-expressing (AtT20) and non-*POMC* expressing (3T3-L1) cell lines. A *tpit* putative promoter region was cloned into a CpG-free vector in order to study the effect of specific CpG methylation.
Results
The region of DNA immediately 5' to the transcription start site of *tpit* is methylated in the non-*POMC* expressing 3T3-L1 cell line, but unmethylated in AtT20 cells. Following chronic glucocorticoid exposure this region becomes *de-novo* methylated in AtT20 cells. A 410 bp region of DNA corresponding to this area has promoter activity as assessed in transient transfection experiments in AtT20 cells using a reporter construct that lacks CpG sites in the backbone. Specific methylation of the seven CpG sites alone in the 'promoter' caused inhibition of expression.
Conclusion
Chronic glucocorticoid exposure causes *de novo* methylation and silencing of *tpit* expression. These data have important implications for use of epigenetic modifying agents after chronic glucocorticoid therapy to allow recovery of the HPA axis.

DOI: 10.1530/endoabs.34.P291

P292

Follow-up, surgery and proton beam therapy for a pituitary sella chondrosarcoma

Yasir Mohamed Elhassan[1], Bernie Foran[1], Tim Hodgson[1], Paul Ince[1,2], Saurabh Sinha[1] & John Newell-Price[1,2]
[1]Sheffield Teaching Hospitals NHS Trust, Sheffield, UK; [2]University of Sheffield, Sheffield, UK.

A 41-year-old woman was referred with 9 months history of secondary amenorrhoea and galactorrhoea. She was otherwise well and not taking any medications. Biochemical evaluation showed prolactin 2000 mU/l but otherwise unremarkable. Pituitary MRI revealed a 30 mm pituitary lesion with right cavernous sinus invasion, presumed to be a craniopharyngioma due to the presence of calcification. Surgical intervention was recommended, but the patient declined. She was commenced on cabergoline with normalisation of prolactin and restoration of menses. Serial pituitary MRI showed stable disease until 3 years later where there was an increase in size and filling of the sphenoid sinus. She developed right 6th cranial nerve palsy. A stronger recommendation for surgery was made, but also declined. A year later, MRI showed a further increase in size

of the pituitary lesion. Right 6th and left 4th nerve palsies were noted. Following further discussion she underwent endoscopic transsphenoidal surgery to debulk the lesion with complete resection of the suprasellar component but with residual tissue within the pituitary fossa and right cavernous sinus. Postoperatively, pituitary function remained normal. Histology revealed WHO grade-2 chondrosarcoma. She was referred for proton beam therapy in Florida, USA, and tolerated the treatment well. Pituitary MRI 3 months later showed reduction of the pituitary fossa and cavernous sinus tumour residues. Her pituitary functions remain intact.

Pituitary chondrosarcomas are rare tumours of cartilage-forming cells arising from the sella, usually presenting as a non-functioning mass. The best predictor of good long-term outcome is the extent of resection of the initial tumour. However, the anatomical location may render complete resection extremely difficult and hazardous to achieve. Our case demonstrates that these tumours are slow growing. It illustrates the importance of histological diagnosis for proton therapy to be offered which is recommended for any residual as it allows safer delivery of higher radiation doses.

DOI: 10.1530/endoabs.34.P292

P293

GH excess of unknown origin

Agnieszka Falinska, Yong Yong Ling, Raj Tanday, Tanaz Vakilgilani & Jeannie F Todd
Imperial Centre of Endocrinology, Hammersmith Hospital, London, UK.

A 68-year-old patient was noted to have prognathism, broad fingers and toes and coarse facial features during her admission for a hip replacement 2 years ago. Her family noted change to her facial features and she admitted to increase in her shoe size over 10 years. Her past medical history included treated hypertension only. Her oral glucose tolerance test (OGTT) confirmed paradoxical rise of GH with peak 6.23 μg/l. Her initial IGF1 was raised at 131 nmol/l (6–30 nmol/l) with normal remaining pituitary function. Her initial MRI showed a normal anterior pituitary, 8 mm lesion in superior–posterior pituitary elevating the pituitary stalk and infundibulum and an incidental finding of sphenoid wing meningiomas. She was referred for transpehnoidal hypophysectomy. The histology showed fibrosis with no evidence of an adenoma.

However her GH burden remained unchanged postoperatively. Post-surgical IGF1 was 114 nmol/l (6–30 nmol/l) and again she failed to suppress GH during OGTT. Ga68 DOTATATE PET CT showed no obvious source of ectopic GH or ectopic GHRH. There was increased uptake within the meningioma which is an expected phenomenon. In addition it demonstrated an 8 mm nodule adjacent to the right lung hilum which was investigated further with high resolution CT and confirmed static nodule with a plan to repeat imaging in 1 year. Repeated CT brain and two brain MRIs confirmed that the posterior–superior pituitary lesion has been excised and the presence of an unchanged two meningiomas but did not reveal any new suspicious lesions within pituitary. She was discussed at local pituitary MDT and the decision was to treat her with somatostatin analogues for 1 year and re-assess.

This patient GH burden could be due to hidden source in pituitary gland or due to ectopic source. Acromegaly caused by either ectopic GHRH or ectopic GH-producing tumours is very rare accounting for $<1\%$ of the cases. Its recognition is clinically important as different therapeutic approaches are required.

DOI: 10.1530/endoabs.34.P293

P294

Hypopituitarism from Hyderabad

Raj Tanday, Agnieszka Falinska, David Woods, Yong Yong Ling, Tannaz Vakilgilani & Jeannie Todd
Hammersmith Hospital, London, UK.

A 33-year-old woman was referred to our service for investigation of secondary amenorrhea. She is from India and moved to the UK 8 years ago. She has two children aged 7 and 4 years. She has a history of TB adenitis treated in 2007 with quadruple anti TB medication for 6 months. She was told she had no pulmonary involvement and was clear after treatment. She is currently on no medication. During 2012 she noticed her menstrual cycles were lengthening with amenorrhoea since January 2013. She also complained of weight loss, abdominal pains and general malaise for several months. She denied significant *post-partum* haemorrhage.

Examination revealed BMI of 26. Cardiorespiratory and GI examination was normal apart from a blood pressure of 99/55. She had no postural drop.

Baseline bloods showed prolactin 74 mU/l, TSH 3.31 mU/l, fT3 <3.31 pmol/l, fT4 <5.2 pmol/l, ACTH 9.7 ng/l, cortisol 20 nmol/l, DHEAS <0.4 μmol/l, LH 1 IU/l, FSH 4.5 IU/l, oestradiol <70 pmol/l, IGF1 6.6 nmol/l.

She was brought back the same evening and commenced on hydrocortisone 10 mg, 5 mg, 5 mg. Steroid rules were explained. At the next visit she reported feeling stronger with more energy. Thyroxine 25 μg was added. It was explained that we will replace other hormones in time. A DEXA scan, USS pelvis, urgent MRI and ITT have been arranged.

The MRI showed an empty sella of normal size with no pituitary tissue, no pituitary mass, normal infundibulum and no cerebral abnormality.

In summary our patient has panhypopituitarism with MRI appearances of an empty sella. We propose the following causes:

1) Pituitary infarction due to undiagnosed/delayed Sheehan's.
2) Previous hypophysitis.
3) TB infection.
4) Idiopathic empty sella.

DOI: 10.1530/endoabs.34.P294

P295

Can 0900 h serum cortisol levels be used to predict patient's response to the insulin tolerance test?

Nikolaos Kyriakakis, Elizabeth Appleton, Julie Andrew & Robert Murray
Leeds Centre for Diabetes and Endocrinology, Leeds Teaching Hospitals NHS Trust, Leeds, UK.

Aim

The insulin tolerance test (ITT) is considered the gold standard test in assessing the integrity of the hypothalamic–pituitary–adrenal (HPA) axis. The aim of this study is to evaluate if the 0900 h cortisol levels can be predictive of the patient's response to hypoglycaemia, minimizing the use of the ITT as it is labour, intensive and unpleasant for the patient.

Methods

This is a retrospective study of 110 ITTs performed at the Endocrine Unit of St James's Hospital in Leeds between January 2010 and July 2013. The baseline serum cortisol level was compared against the patient's peak cortisol response to hypoglycaemia (normal ≥ 500 nmol/l; suboptimal ≥ 400 nmol/l but <500 nmol/l; and insufficient <400 nmol/l).

Results

Six patients had basal cortisol <100 nmol/l. All of them (100%) failed to respond to hypoglycaemia. 57 had basal cortisol between 100 and 299 nmol/l. Among them, 22 (38.6%) had a normal ITT, 18 (31.6%) had a suboptimal response and 17 (29.8%) had an inadequate response to hypoglycaemia. The remaining 47 patients had baseline cortisol ≥ 300 nmol/l, with 32 of them (68.1%) responding normally, 14 (29.8%) having a suboptimal response and one patient (2.1%) failing the ITT. The Pearson's correlation between basal and peak cortisol was calculated at 0.685.

Conclusions

Measurements of basal serum cortisol can identify patients for whom the ITT may not be necessary. Basal cortisol <100 nmol/l indicated a HPA axis insufficiency, whereas levels ≥ 300 nmol/l suggested an at least suboptimal response to hypoglycaemia, which would allow the use of steroids in the context of an acute illness only. In both groups the ITT could be avoided, as it would not alter patients' management. On the contrary, baseline cortisol between 100 and 299 nmol/l cannot be used to predict patient's response to hypoglycaemia, therefore dynamic tests to assess the HPA axis integrity should be performed.

DOI: 10.1530/endoabs.34.P295

P296

The challenge of diagnosing Langerhan cell histiocytosis as the cause of a hypothalamic lesion presenting with diabetes insipidus

Muditha Weerakkody[1], Saurabh Sinha[2] & Richard Ross[1]
[1]University of Sheffield, Sheffield, UK; [2]Sheffield Teaching Hospitals NHS trust, Sheffield, UK.

Langerhan cell histiocytosis (LCH) is a rare; incidence 1.8/100 000. It affects bone, skin, and pituitary but can involve any organ. Diabetes insipidus (DI) is reported in 15–50% of patients, and anterior pituitary dysfunction in 5–20%. We describe a patient whose diagnosis was delayed because of the challenge in making a tissue diagnosis.

A 42-year-old female presented in 2010 with sudden onset deafness and vertigo then 1 year later developed DI. Brain MRI showed absence of posterior pituitary bright spot, thickened pituitary stalk but no other lesion. Anterior pituitary function was normal except for hypogonadotrophic hypogonadism. She was commenced on desmopressin, with a good response. No diagnosis was made and surgery was not indicated. Twelve months later, she was tired and unwell, central hypothyroidism, and GH deficiency was diagnosed and replaced. Her MRI remained unchanged. Screening for tuberculosis, sarcoid, germinoma, skeletal survey, and bone marrow were normal. Six months later she complained of left arm, leg weakness and was admitted with obstructive jaundice. An MRI demonstrated focal areas of abnormal signalling within multiple bones and vertebrae, a mass in the thyroid and obstruction of common bile duct. An iliac bone biopsy showed infiltration with a mixed population of histiocytes staining for CD1a, CD45 and S100, suggestive of LCH. ^{18}F-fluoro-2-deoxyglucose positron emission tomography (FDG-PET) CT demonstrated areas of abnormal activity in most of skeleton and low grade activity in pituitary, thyroid, and pancreas. A diagnosis of multisystem LCH was made. The biliary stricture was stented, she was treated with Vinblastine but her disease progressed and she was commenced on cyclical chemotherapy with cytarabine.
This case illustrates the complexity of diagnosing LCH, especially with the necessity of histology for definitive diagnosis and highlights that when LCH is considered screening is probably required with FDG PET.

DOI: 10.1530/endoabs.34.P296

P297

Just another 'incidental finding'?

Sumithra Giritharan, Tara Kearney, Helen Doran & Kanna Gnanalingham
Salford Royal NHS Foundation Trust, Salford, UK.

A 60-year-old female was referred to the department in October 2010 after a CT thorax revealed an incidental finding of a right adrenal nodule. On questioning, the patient reported some sweats but otherwise no other symptoms of hormonal excess. Adrenal functional studies were normal and it was therefore decided that the patient should have a repeat CT scan in 6 months. Repeat CT adrenal glands in April 2011 did not show any changes to the size of the nodule but gave indeterminate washout characteristics. It also flagged up an incidental finding of bilateral renal cysts and recommended imaging with ultrasound. This revealed simple renal cysts but an incidental finding of a renal stone in the lower pole of the left kidney. Patient was referred to the urology team who then made a note of an incidentally raised serum calcium level. Patient was therefore re-referred back to endocrine clinic where her biochemistry was consistent with primary hyperparathyroidism. Localisation studies did not show concordance and therefore patient was offered bilateral neck operation which she refused. Coincidentally, baseline pituitary function tests were carried out although the indication for this was unclear. Surprisingly, this revealed a raised IGF1 value 57.2 nmol/l (9.8–27.6 nmol/l). Patient then proceeded to have an oral glucose tolerence test which revealed a GH nadir of 1.6 μg/l. She was otherwise eupituitary. MR scan of the pituitary revealed a small 7 mm right sided microadenoma. Patient proceeded to have endoscopic transphenoidal surgery. Post-operative testing revealed biochemical cure with a GH nadir of 0.1 μg/l on oral glucose tolerance test and a normalized IGF1 value 155 ng/ml (41–168). MEN genetic screening was negative and screening fasting gut peptides were also negative.

DOI: 10.1530/endoabs.34.P297

P298

Could GH deficiency exacerbate insulinoma?

Tannaz Vakilgilani, Raj Tanday, David Woods, Agnieszka Jeannie, Young Young Ling & Jeannie F Todd
Imperial Centre of Endocrinology, Hammersmith Hospital, London, UK.

A 53-year-old retired military soldier had a 9 months history of recurrent blackouts predominately associated with blurred vision, feeling shaky, sweaty and they were mainly before meal. His past medical history included asthma, multiple shrapnel injuries, hearing impairment secondary to blast injury. He was on atorvastatin, ompeprazole and inhalers.
He was admitted acutely to his local hospital and blood glucose 0.6 mmol/l was found. Prolonged 72 h fast showed an elevated C-peptide and insulin in the context of hypoglycaemia and negative sulfonylurea (SU) screen consistent with a diagnosis of insulinoma. Subsequent CT abdomen revealed a 14 mm hypervascular lesion within the body of the pancreas. He was then referred to our outpatient endocrine clinic.
He experienced the above symptoms with normal capillary blood glucose (CBG) in clinic but was admitted for completion of investigations. He had a normal EEG and CT brain. Repeat prolonged fasting confirmed hypoglycaemia with blood glucose of 1.7 mmol/l at 52 h with inappropriately elevated C-peptide of 1396 pmol/l and insulin of 21.6 mU/l and negative SU screen. I addition, concomitant plasma GH of 1.93 μg/l (inappropriately low in context of hypoglycaemia) but normal cortisol of 651 nmol/l. LH, FSH, and testosterone were 3 IU/l (2–12), 3.3 IU/l (1.7–8), and 6.9 nmol/l (10–30) respectively which is suggestive of secondary hypogonadism. The reminder of a pituitary profile was normal.
Gallium 68 DOTOTATE PET–CT scan confirmed gallium avid lesion in the body of the pancreas and EUS also confirmed a lesion.He is currently waiting for formal ITT to confirm GH deficiency and has commenced testosterone replacement. He has been referred to surgeons for laparoscopic resection of the lesion.
In this case previous blast injury could be the cause of secondary hypogonadism and GH deficiency. It raises the possibility that elimination of GH counter-regulatory action in hypoglycaemia could have exacerbated insulinoma.

DOI: 10.1530/endoabs.34.P298

P299

Pituitary tuberculosis

Darshi Sivakumaran, Gideon Mlawa & Gul Bano
St George's Healthcare NHS Trust, Tooting, London, UK.

Tuberculosis affecting the pituitary gland is a rare phenomenon and is usually the result of haematological spread from another site. Only 0.9% of cases of TB in the UK, in 2012, showed any CNS involvement, excluding meningitis[1]. The clinical presentation of pituitary TB, range of hormonal dysfunction and radiological features can vary, making it hard to identify the condition. We report two clinical cases seen in our department.
Case 1
A 48-year-old female from Somalian, was referred with a 7-month history of lethargy and blood tests suggesting secondary hypothyroidism. Subsequent endocrine investigations confirmed panhypopituitarism and diabetes insipidus. An MRI pituitary showed the pituitary gland to be bulky with loss of the posterior pituitary bright spot and increased signal in the lower end of the stalk. CT chest showed no mediastinal or hilar lymphadenopathy, however, 2.5 cm right axillary lymphadenopathy was seen. An ultrasound guided FNA was performed; TB culture isolated acid fast bacilli and PCR confirmed the presence of *Mycobacterium tuberculosis* complex.
Case 2
A 40-year-old female from Cameroon, was referred whilst receiving inpatient treatment for multi-drug-resistant TB with blood tests suggestive of secondary hypothyroidism. Imaging performed for a new right CN VI palsy showed a bulky pituitary gland when reviewed retrospectively. The remainder of her pituitary profile was normal. Her thyroid function tests normalised with ongoing TB treatment, as did her radiological features.
The incidence of tuberculosis in London, in 2012, was much higher than any other capital in Western Europe (41.8 cases/100 000 population)[1]. Previous case reports have shown a delay in identifying pituitary TB can lead to unnecessary surgical intervention and more permanent endocrinopathies. We must exercise vigilance and consider the diagnosis early, especially in high risk groups.
References
1. *Tuberculosis in the UK: 2013 Report – Public Health England.*

DOI: 10.1530/endoabs.34.P299

P300

Immunohistochemical features of PANCH tumour, a mixed pituitary adenoma/gangliocytoma, a rare cause of acromegaly

Leong Quah[1], Ute Pohl[1], Jonathan Pollock[1] & James Ahlquist[1,2]
[1]Queen's Hospital, Romford, Essex, UK; [2]Southend Hospital, Westcliff on Sea, Essex, UK.

PANCH tumour (pituitary adenoma with neuronal choristoma), is a very rare form of pituitary pathology composed of a mixed pituitary adenoma/gangliocytoma. We describe a patient with acromegaly who had evidence of GH synthesis in the neuronal component of a PANCH tumour. A 55-year-old woman was found to have facial features of acromegaly, confirmed biochemically: basal GH 13.56 ng/ml, GTT nadir 8.87 ng/ml, and IGF1 raised at 97.2 nmol/l (ref 9–40). Pituitary function was otherwise normal. MRI revealed a 25 mm mass in the

pituitary fossa, with cavernous sinus extension; there were no unusual radiological features. She underwent endoscopic trans-sphenoidal surgery, during which the tumour was noted to have an unusual, slightly fibrous consistency. Intra-operative histological examination suggested that the tumour may be a ganglioglioma. Following surgery IGF1 had fallen to 273 ng/ml (ref 99–254) and pituitary function remained intact.
Histological examination of the tumour revealed islands of pituitary adenoma embedded in a neuropil substrate made up of ganglion-like cells, some of which showed dysplastic features (cytomegaly, binucleation, and dysmorphism). Ganglion cells were embedded within both the adenoma and the neuroglial tissue. Immunohistochemistry confirmed that the adenoma cells were positive for GH and prolactin, with scattered cells positive for TSH also. In addition, a subpopulation of the ganglion cells also showed strong staining for prolactin, and weak staining for GH, TSH, and ACTH. Both pituitary adenoma and ganglion cells were strongly positive for synaptophysin; chromogranin only stained rare small cells. Glial fibrillary acid protein staining was generally negative throughout. The neural tissue features were described as typical of a neuronal choristoma.
The combination of a pituitary adenoma with neuronal choristoma (PANCH) is a very rare form of pituitary pathology. The finding of neuronal tissue expressing GH and prolactin in association with a somatotroph adenoma is intriguing. The pathogenesis of this phenomenon is not clear. The combination of two distinct GH and prolactin-positive cell types, pituitary adenoma and gangliocytoma, occurring within a single tumour, suggests that the neuronal cell population may have arisen as a result of neuronal differentiation within a pituitary adenoma.

DOI: 10.1530/endoabs.34.P300

P301

Sheehan's syndrome: end of 15 years of hardship

Akshatha Taranath Kamath & Sampath Satish Kumar
Narayana Hospitals, Bangalore, Karnataka, India.

Introduction
Sheehan's syndrome is hypopituitarism occurring due to pituitary infarction secondary to severe *post-partum* haemorrhage. Here, we are presenting an interesting case history of Sheehan's syndrome diagnosed 15–20 years after its onset.
Case history
A 47-year-old lady attended emergency room (ER) with recurrent episodes of nausea, vomiting and dizziness on standing. Initial evaluation revealed hypotension and the random serum cortisol was 0.22 mg/dl. Adrenal insufficiency was diagnosed and suspected to be due to systemic tuberculosis diagnosed a few months ago. She was started on oral hydrocortisone and discharged from ER.
She returned to endocrinology OPD 10 months later with similar symptoms. PMH revealed that she delivered her last child at home. She had severe bleeding during and after delivery. Lactation was impaired and she remained amenorrhoic since then. Biochemistry revealed ACTH 3.63 pg/ml (7.2–63.3), FT_4 0.31 ng/dl (0.89–1.76), TSH 6.51 μIU/ml (0.35–5.5), LH 3.91 mIU/ml (8.0–33.0), FSH 9.9 mIU/ml (17–95), prolactin 4.3 ng/ml (5.0–35), sodium127 mEq/l (135–150), potassium3.7 mEq/l (3.5–5.0), hemoglobin 9.5 gm/dl, and 0800 h cortisol 0.67 μg/dl (4.30–22.40). MRI scan of the pituitary revealed partial empty sella. She was diagnosed to have pan-hypopituitarism. She has been started on oral hydrocortisone, thyroxin and HRT was deferred because of menopausal age.
Conclusion
The prevalence of Sheehan's syndrome has reduced significantly in developed countries but is still prevalent in developing countries. Adrenal insufficiency was the presenting symptom in this patient. As she had been diagnosed with tuberculosis, the adrenal insufficiency was thought to be primary and not due to pituitary cause. But with further probing and detailed history taking, the underlying cause and associated problems were found. Detailed history taking and high index of suspicion for pituitary causes are still the most important aspects of clinical practice.

DOI: 10.1530/endoabs.34.P301

P302

Primary polydipsia in a family with a known mutation in the AVP gene

Ruth Casey[1], Anne Marie Hannon[1], Caroline Joyce[2], Susan O'Connell[3] & Domhnall O'Halloran[1]
[1]Department of Endocrinology, Cork University Hospital, Cork, Ireland; [2]Department of Biochemisty, Cork University Hospital, Cork, Ireland; [3]Department of Paediatric Diabetes and Endocrinology, Cork University Hospital, Cork, Ireland.

Diabetes insipidus is characterised clinically by the inappropriate production of large volumes of dilute urine, even in the presence of clinical dehydration or depravation of water. DI occurs either due to deficiency or insufficiency of arginine vasopressin (AVP) hormone production. The gold standard test remains the water deprivation test. Hereditary DI accounts for $<10\%$ of all cases.
We present a family with a known heterozygous missense mutation, c232> A(GLU78LYS) in the AVP gene. The mother has biochemically confirmed central DI and the mutation. She has four children. Three of whom have been tested. The eldest daughter, who was symptomatic, tested positive for the mutation and also failed the water deprivation test, on MRI imaging she had absence of the bright spot in the posterior pituitary. The next daughter, also tested positive for the mutation, however although she was symptomatic she passed the water deprivation test. The third child is asymptomatic, does not have the mutation and passed the water deprivation test. The fourth child has yet to be tested.
This case illustrates that psychogenic polydipsia can co-exist in families, in whom a diagnosis of familial diabetes insipidus has already been established in other family members. A child exhibiting water seeking behaviour, can mistakenly be assumed to suffer from the same condition. Caution is required before prompt diagnosis, as there is a risk from hyponatraemic seizures with inappropriate desmopressin use.
However, primary polydipsia, does not preclude this child from developing diabetes insipidus in the future and genetic analysis still has an important role to play in identifying those at risk of developing DI in the future.
This case demonstrates the complex nature of diagnostic and management issues when faced with symptoms and a positive genetic test in the face of negative biochemistry.

DOI: 10.1530/endoabs.34.P302

P303

Differential p120 isoform distribution and splicing regulator ESRP1 expression distinguishes sparsely and densely granulated somatotroph adenomas

Sarah Larkin & Olaf Ansorge
University of Oxford, Oxford, UK.

Somatotroph adenomas (SA) causing acromegaly are histologically classified into densely (DG) and sparsely granulated (SG) subtypes. We[1] and others have shown that these histological subtypes may be clinically relevant. In short, SGSAs are generally found in younger, mainly female patients. They are larger at presentation, more invasive and show higher proliferation indices compared with DGSAs. Fibrous bodies (FBs) are the histological hallmark of SGSAs; they consist of accumulated cytokeratins, implying loss of normal cytoskeletal architecture and disrupted membrane attachment in SGSAs. The mechanisms linking FB formation with a more invasive phenotype are not known. Here we tested the hypothesis that a regulator of adherens junction integrity, p120, is associated with FB formation. We conducted a comprehensive assessment of the adherens junction complex by immunohistochemistry and found that total loss of membranous E-cadherin and α-, β- and γ- catenin in SGSAs is associated with redistribution of p120 from the membrane to FBs. Alternative splicing of p120 leads to short (epithelial) and long (mesenchymal) variants. Colocalisation experiments showed that the short, but not the long p120 isoform was found at FBs. Expression (mRNA and protein) of the two isoforms remained unchanged; however, ESRP1 mRNA (a splicing regulator that promotes the epithelial p120 variant) was expressed 80-fold higher in DGSAs than SGSAs. It is hypothesised that sequestration of the short isoform of p120 at the FB inhibits function in SGSA cells, mimicking aspects of epithelial-to-mesenchymal transition, a common phenomenon associated with a more invasive phenotype in neoplasms. Further characterisation of this mechanism in SGSAs may reveal novel pharmacologic targets for the treatment of SGSAs that are resistant to current therapy.
References
1. Larkin *et al*. *Eur J Endocrinol* 2013 **168** (4) 491–499.

DOI: 10.1530/endoabs.34.P303

P304

Disordered sleep architecture is a common finding in acromegaly

Andrew S Powlson[1], Laksha Bala[1], Anand K Annamalai[1], Olympia Koulouri[1], Alison Webb[1], Samantha Moir[2], John M Shneerson[2] & Mark Gurnell[1]
[1]Metabolic Research Laboratories, Wellcome Trust–MRC Institute of Metabolic Science, University of Cambridge, Cambridge, UK; [2]Respiratory Support and Sleep Centre, Papworth Hospital, Papworth Everard, UK.

Sleep disordered breathing (SDB), including obstructive sleep apnoea (OSA), is associated with excessive daytime somnolence, and impacts significantly on quality of life in affected individuals. It also predisposes to premature cardiovascular (hypertension, congestive cardiac failure, myocardial infarction, sudden death, and stroke) and metabolic (diabetes mellitus and dyslipidaemia) dysfunction.

SDB is a well-recognised complication of acromegaly. In most centres, routine screening for SDB consists of an Epworth sleepiness scale (ESS) ± overnight oximetry to measure desaturation index (DI). However, polysomnography remains the gold-standard investigation. Here, we present the largest study to date of SDB in newly diagnosed, treatment naïve, patients with acromegaly ($n=39$) using polysomnography.

OSA, defined by the apnoea–hypopnoea index (AHI), was a common finding (77%) in newly-diagnosed acromegaly: mild OSA, $n=12$; moderate OSA, $n=5$; and severe OSA, $n=13$. However, in contrast DI markedly underestimated the extent of sleep disordered breathing in our cohort: mild OSA, $n=11$; moderate OSA, $n=7$; and severe OSA, $n=3$. ESS also failed to predict the presence of SDB in many patients (ESS >11, $n=12$).

Consistent with the finding of a high rate of OSA, patients exhibited an increased arousal index, with marked disruption of the normal sleep cycle, despite the majority ($n=33$) exhibiting a normal total sleep period time. Twenty-six patients spent longer than predicted in stage 1 sleep, with reciprocal attenuation of the deeper sleep stages (reduced stage 2, $n=25$; reduced slow wave sleep, $n=25$; and reduced REM sleep, $n=30$).

Our findings suggest that the majority of patients with acromegaly have evidence of sleep disordered breathing, with detrimental effects on sleep architecture – in particular marked attenuation of the deeper sleep stages. Moreover, we have shown that the use of ESS and DI as primary screening for SDB in acromegaly underestimates the prevalence of sleep apnoea and may lead to failure to diagnose and treat this important complication.

DOI: 10.1530/endoabs.34.P304

P305

The effects of kisspeptin-54 administration on GH, prolactin, and TSH secretion in healthy women

Shakunthala Narayanaswamy[1], Channa Jayasena[1], Alexander Comninos[1], Sanjana Bhalla[1], Ali Abbara[1], Zainab Gainyu-Dada[1], Mark Busbridge[2], Mohammad Ghatei[1], Stephen Bloom[1] & Waljit Dhillo[1]
[1]Imperial College, London, UK; [2]Imperial College Healthcare NHS Trust, London, UK.

Background

The peptide hormone kisspeptin is essential for human reproduction, acting on the hypothalamus to stimulate GnRH secretion. Kisspeptin is emerging as a possible novel therapeutic for women with infertility. However animal studies suggest that kisspeptin may also stimulate pituitary secretion of GH, prolactin, and TSH, which has important implications for its safety. There has been no previous study investigating kisspeptin effects on non-reproductive hormones in humans.

Aim

To determine whether kisspeptin-54 modulates GH, prolactin, and TSH secretion in healthy women.

Study

This was a prospective, single-blinded placebo-controlled one-way cross-over study. Five healthy women received twice daily s.c. injections of vehicle (saline) from day 7 of their menstrual cycle (follicular phase) for 7 days in month 1 of the study. This was followed in month 2 by twice daily s.c. injections of kisspeptin-54 (6.4 nmol/kg) from days 7 to 14 of their menstrual cycle. Serum samples were analysed *post-hoc* for GH, prolactin, and TSH.

Results

Mean serum GH, PRL, and TSH did not change during the first 4 h following kisspeptin-54 injection when compared with vehicle. The mean frequency or amplitude of GH pulses (which influence GH function) did not change acutely following kisspeptin-54 injection when compared with vehicle. No chronic changes in serum GH, PRL, or TSH were observed over the 7 day period of twice-daily kisspeptin-54 injections when compared with vehicle.

Conclusion

We report that kisspeptin-54 does not acutely or chronically stimulate GH, prolactin, or TSH secretion in healthy women, at a dose which potently stimulates gonadotrophin release. This has important implications for the development of kisspeptin as a potential therapeutic for patients with infertility.

DOI: 10.1530/endoabs.34.P305

P306

One year pituitary function follow-up in survivors of severe brain injury presenting at a regional neurosurgical centre

Zaka Haq, Ioana Onac, Suad Elyas, Sarah Leeder & Trever Wheatley
Princess Royal Hospital/Hurstwoodpark Neurosciences centre, Haywards Heath, UK.

Introduction

Severe brain injury is usually sustained as a result of road traffic accident or high impact fall. Initial presentation of patients depends upon the severity of brain injury. Survivors of severe brain injury carry significant morbidity and often need prolonged period of rehabilitation. About one third of these patients are suspected to have partial or complete pituitary dysfunction which can manifest insidiously many months after the initial insult. Recognition and treatment of pituitary dysfunction is very important for the initial recovery, long-term health, quality of life and general wellbeing.

Method

At Hurstwood Park Neurosurgical Centre, we studied 40 patient records that presented over a period of 3 years from 2010 to 2012 and followed them up for at least one year to look for any evidence of their pituitary function tests performed either in the hospital or in the community. Severe brain injury for the purpose of this study was defined on the basis of Glasgow Coma Scale less than 8 on presentation combined with radiological evidence of brain injury/haemorrhage with or without skull fracture. Information was collected from hospital electronic database, pathology department and by contacting primary care for tests performed elsewhere. Pituitary function tests were followed at 7 days, 3, 6 and 12 month intervals from initial injury.

Results

Out of the 40 patients, 4 died during admission and remaining 36 patients had an average length of stay in hospital of 70.5 days (minimum 12, maximum 266 days).

3 patients (7.5%) had all the pituitary function tests performed at some point in time but only one (2.5%) was followed through the year. At 3 months one patient had IGF1, TFTs and cortisol, 2 had cortisol and TFTs, 4 had TFTs only out of which 3 had repeat TFTs at one year. The acute cortisol (1–7 days) was assessed only for 7 patients (17.5%).

Conclusion

None of the above patients had a structured pituitary or endocrine follow-up and more than two thirds of patients did not have any sort of pituitary function assessment at any time during one year period following injury. Some of these patients may have unrecognised pituitary dysfunction. Currently there are no national guidelines on the management and follow-up of survivors of severe brain injury with regards to their pituitary health and this study stresses the need for the development of such guidelines.

DOI: 10.1530/endoabs.34.P306

P307

The incidence of hypopituitarism in post interventional subarachnoid haemorrhage (SAH) survivors in a tertiary neurosurgical unit

Stephen J McGlynn[1,2], Joanna Cox[1,2], Sumi Giritharan[1,2], Kanna Gnanalingham[1,2], David Hughes[1,2], Russell Sheldrick[1,2] & Tara M Kearney[1,2]
[1]University Hospitals of South Manchester NHS Foundation Trust, Manchester, UK; [2]Salford Royal Foundation Trust, Salford, Greater Manchester, UK.

Background

SAH is a significant cause of morbidity and mortality. Survivors report long term psychological distress, sleep disturbance, libido changes and fatigue. Previous studies describe an increased incidence of hypopituitarism.

Hypothesis
Evaluation of psychological symptoms and clinical and radiological features of SAH could predict the incidence of hypopituitarism.
Patients and Methods
102 post-interventional SAH survivors ((76 male) mean age (range) 50.7 (22–72) years) were studied. Mean time from ictus 21.2 (minimum 12) months. Patients had basal pituitary and glucagon stimulation testing and completed Hospital Acquired Depression Scale (HADS), Davidson Trauma Score (DTS) and WHO Quality of Life (WHOQoL) questionnaires. Radiologic features at presentation (location, severity, Fisher grade) and clinical features (WFNS-SAH grade, intervention modality, presence of hydrocephalus, EVD insertion) were recorded.
Results
49 of 102 participants had pituitary insufficiency. 32 had GH peak below 9 ng/ml post 1 mg s/c Glucagon. 23 had severe GH deficiency (peak <3 ng/ml).
There was no increased incidence of anxiety, depression or post-traumatic stress disorder (PTSD) in the GH deficient cohort.
Site of aneurysm and Fisher grade did not predict the incidence of GHD. Neither clinical assessment of severity at presentation nor development of hydrocephalus requiring an EVD correlated with incidence of GHD.
There was an increased incidence of hypopituitarism in the endovascular coiled (EV) cohort vs surgical clipping.
Discussion
These results suggest that the incidence of hypopituitarism in this population is high (48%) and the incidence of severe GHD (NICE treatment cut-off) was 22.5%. Further studies are required to determine the benefit of replacement in these patients. While there was no increased incidence of psychological morbidity, GH replacement may improve these symptoms in the GHD group.
The higher incidence of hypopituitarism in EV vs clipping may be explained in part by the higher number of EV (79 vs 23) but requires further evaluation.

DOI: 10.1530/endoabs.34.P307

P308

Inhibition of lipolysis improves peripheral and hepatic insulin sensitivity and restores first phase insulin response in patients with acromegaly

Stephen J McGlynn[1], Les Bluck[2], Burak Salgin[3], Peter J Trainer[1], Steve Williams[4] & Claire E Higham[1]
[1]The Christie NHS Foundation Trust, Manchester, UK; [2]MRC-Human Nutrition Research, Cambridge, UK; [3]Deparment of Paediatrics, Addenbrookes Hospital, Cambridge, UK; [4]University of Manchester, Manchester, UK.

Acromegaly causes impaired insulin sensitivity and reduced fat mass. Using acipimox to block lipolysis, the impact of free fatty acids (FFA) on insulin sensitivity in active acromegaly was investigated. ^{1}H MRS was used to quantify triglyceride (TG) content of liver and muscle.
Methods
6 patients with active acromegaly (AA) (5M, age 59 (34–70), IGF-I (ng/ml) (median (range)) (452 (342–1002)) were studied on 2 visits; (i) baseline (BL) (ii) after Acipimox (WA) 250 mg 4 hourly 2000–0800 h. Using stable isotopes of glucose allowed insulin sensitivity assessment under basal conditions overnight (ON). Minimal modeling (MINMOD) of a frequently sampled IVGTT provided estimations of insulin sensitivity (Si), glucose effectiveness (Sg), acute insulin response to glucose (AIRg) and disposition index (DI).
8AA (6M, age 39.5 (23–66), IGF-I (ng/ml) 541 (326–1244) and 5 healthy volunteers (HV) (3M age 30.5 (28–39)) had liver, gastrocnemius (GN) and tibialis anterior (TA) TG content estimated by ^{1}H MRS.
Results
Basal: Mean ON insulin (AUC(s.d.) mU h per l) was lower with Acipimox (WA) vs Baseline (BL) (135 (52) vs 279 (97), $P=0.04$). Mean ON glucose (AUC (± 2 s.d.) mmol h per l) was lower WA vs BL (52 (4) vs 63 (9), $P=0.03$). Hepatic insulin sensitivity (HIS) higher in all WA vs BL (min l/mmol per pmol $\times 10^2$) (16.3 (8.3) vs 8.4 (3.3), $P=0.02$).
Stimulated: Si (10^{-4} ml/uU per min) was higher WA vs BL in 4 of 5 successful models, (mean(s.d.)) (14.1 (18.2) vs 16.7 (11.9), $P=$NS); Sg (per min $\times 10^3$) no difference observed WA vs. BL (11.3 (4.3) vs 12.2 (3.2), $n=5$, $P=$NS); AIRg (uU/ml min) improved WA vs BL (66.3 (46.2) vs 40.1 (33.7), $n=5$, $P=0.01$). DI ($\times 10^3$) improved WA vs BL (98.5 (74) vs 45.2 (56.6), $n=5$, $P=0.01$).
Liver TG:Water ratio was lower in AA than HV; median(range) (0 (0–0.05) vs 0.12 (0.02–0.22), $P=0.01$).
Conclusion
These results suggest that lipolysis is a key factor in impaired insulin sensitivity in acromegaly. Inhibiting lipolysis (decreasing FFA production) improves peripheral and hepatic insulin sensitivity, and restores 1st phase insulin secretion. The low hepatic fat observed suggests that FFA flux may be acting as a substrate for increased hepatic glucose production, which is reduced by lipolysis inhibition.

DOI: 10.1530/endoabs.34.P308

P309

Acromegaly in a treated prolactinoma

Funke Akiboye, Adam Nicholls & Triston Richardson
Royal Bournemouth Hospital, Bournemouth, UK.

A 55-year-old male was referred to the endocrine clinic in October 2006 with a 6 month history of reduced libido, lethargy and right sided retro-orbital headaches. He was otherwise well and not on any medications. Examination revealed gynaecomastia, but no other cutaneous stigmata of endocrinopathies and visual fields were full to confrontational testing.
Prolactin was elevated to 4791 mU/l, and a subsequent MRI confirmed a macroadenoma measuring 8×11 mm, with no compression of the optic chiasm. The rest of the anterior pituitary profile was normal, including IGF1. Carbergoline treatment at a dose of 250 μg twice per week provided a good biochemical and clinical response. In view of new symptoms (skin thickening and a change of ring size), further endocrine testing was performed, which demonstrated a raised IGF1, and acromegaly was confirmed with glucose suppression testing. MRI scans showed no change in the original adenoma. Subsequent monitoring of his IGF1 rising levels and he underwent hypophysectomy, with glucose suppression testing post-operatively confirming cure.
There have been a few reports of the development of co-secretion in patients with initial prolactin-only secreting adenomas. Annual screening has been suggested although this is expensive, and may not always be warranted. An open mind to the development of acromegaly should be kept particularly in light of new symptoms in patients with prolactinomas.

DOI: 10.1530/endoabs.34.P309

P310

The effect of somatostatin analogues on the hypothalamo-pituitary-thyroid axis and peripheral thyroid hormone dependent tissues in patients with thyrotropin secreting pituitary tumours

Olympia Koulouri[1,2], Carla Moran[1,2], Andrew Powlson[1,2], Nagui Antoun[2], Heok Cheow[2], Andrew Hoole[2], David Halsall[2], Krishna Chatterjee[1,2] & Mark Gurnell[1,2]
[1]University of Cambridge, Cambridge; UK, [2]Addenbrooke's hospital, Cambridge, UK.

Background
Thyrotropin secreting adenomas (TSHoma) are considered to be rare pituitary tumours. However, improvements in imaging techniques and greater use of more sensitive thyrotropin (TSH) assays has led to a recent increase in the detection rate of TSHomas and, specifically, the identification of more microadenomas. Surgery is considered the mainstay of treatment, however, primary medical therapy with somatostatin analogues is an emerging alternative therapeutic option.
Methods
10 patients with TSHomas were studied at baseline and 3 months after starting long-acting somatostatin analogues. Investigations included measurement of thyroid function tests, metabolic parameters and a panel of biomarkers of thyroid hormone action. Body composition was assessed using Dual-energy X-ray absorptiometry (DXA), and Whole Body Resting Energy Expenditure (REE) measurement was performed using a ventilated hood indirect calorimeter the morning after an overnight fast. Participants were fitted with an 'Actiheart' device to allow estimation of average sleeping heart rate. Pituitary imaging was performed using volume MRI and PET-CT.
Results
Free thyroid hormones normalized in 8 patients. TSH either normalized ($n=6$) or became frankly suppressed ($n=4$), with two patients requiring supplementation with levothyroxine as part of a 'block and replace' regimen. Significant changes in creatine kinase ($P<0.05$), sex hormone binding globulin ($P<0.05$) and total cholesterol ($P<0.05$) were observed, together with a significant reduction in REE ($P<0.005$) and sleeping heart rate ($P<0.05$). The majority of patients ($n=7$) had either osteopaenia or osteoporosis at baseline. Reduction in tumour size was only observed in 2 cases, but PET tracer uptake diminished in 8.

Conclusion
Somatostatin analogues are very effective in controlling hyperthyroidism in patients with TSHomas, leading to favourable changes in thyroid hormone dependent target tissues, such as heart, muscle, and liver. Unlike in patients with acromegaly, tumour size does not change significantly after 3 months of treatment in the majority of patients.

DOI: 10.1530/endoabs.34.P310

P311

A medically managed pituitary tumour and ovarian tumours in a young female

Muditha Weerakkody[1] & Prasad Katulanda[2]
[1]The National Hospital of Sri Lanka, Colombo, Sri Lanka; [2]Faculty of Medicine, University of Colombo, Colombo, Sri Lanka.

Anterior pituitary hyperplasia is known to occur with primary hypothyroidism mimicing a pituitary tumour. Massive ovarian enlargement is sometimes associated with juvenile primary hypothyroidism but rarely reported in adults. We report a case of a 23-year-old female who presented with features of a pituitary macroadenoma and bilateral ovarian enlargement which regressed completely with thyroxine therapy.
A 23-year-old woman presented with nipple discharge, irregular menstruation, weight gain and progressive abdominal distension. On examination she had galactorrhoea, coarse dry skin and a firm pelvic mass. Visual fields were normal. Serum prolactin was 227 ng/dl (1.9–25), TSH $>$ 100 mIU/l (0.3–4.2) and free T4 2.9 ng/dl (5.1–14.1). MRI pituitary showed a lobulated intrasellar mass with suprasellar extension suggestive of a pituitary macroadenoma. Ultrasound pelvis revealed multiloculated thin septal cystic lesions in both adenexial regions with no separate ovarian tissue identified.
Profound hypothyroidism accounting for the above findings was considered and she was commenced on levo thyroxine 100 mic/dl. Her symptoms progressively resolved with time. Thyroid function tests were normalized after 3 months and prolactin after 8 months. MRI scan pituitary returned to normal within 8 months and ultrasound pelvis within 6 months.
Pituitary hyperplasia is known to occur in primary hypothyroidism due to hyperplasia of both thyrotropes and lactotropes caused by TRH stimulation. This may appear as a 'pitiutary pseudotumour'. Massive cystic ovarian enlargement with primary hypothyroidism is commoner in longstanding juvenile hypothyroidism, due to unusually high levels of TSH which have weak FSH like activity. This case illustrates how pituitary enlargement mimicking a macroadenoma and massive ovarian enlargement can occur with profound hypothyroidism with complete regression following thyroxine therapy. Awareness that ovarian and pituitary enlargement can be caused by severe hypothyroidism may spare patients from unnecessary surgical interventions.

DOI: 10.1530/endoabs.34.P311

P312

Isolated 6th nerve palsy, a surprising complication of acromegaly

Sriranaganath Akavarapu, Anna Pouncey, Ali Alexandra de Juniac & Jeremy Cox
St Mary's Hospital, Imperial Collage NHS Trust, London, UK.

Background
It is estimated that 1-6% of patients with a pituitary adenoma develop ocular nerve palsies. These are primarily due to tumour extension into the cavernous sinus and most commonly affect the 3rd cranial nerve. Because of its sheltered position within the sinus, the 6th cranial nerve is rarely affected. When this does occur, it most commonly results from ischemic neuropathy secondary to diabetes, hypertension or mononeuritis multiplex.
Case report
A 35-year-old male presented with a one-month history of double vision on looking left. He described symptoms as worse in the mornings, particularly after a night of sleeping on his left-hand side. Formal visual field examination showed no field defects, but a left 6th cranial nerve palsy was clearly demonstrated. No other neurological defects were elicited and physical examination was unremarkable save for enlarged hands and frontal bossing. A diagnosis of acromegaly was confirmed with an oral glucose tolerance test which showed failure to suppress growth hormone below 0.3 mcg/L. IGF-1 levels were measured at 109 nmol/L. Further testing revealed an impaired fasting glucose with an Hba1c of 6.2% and a short synacthen test showed an inadequate rise in the cortisol response. Other pituitary functions were unimpaired and an autoimmune screening was negative. ESR was within the normal range. An MRI of the pituitary revealed a large non-enhancing sellar lesion with marked extension into the left parasellar territory. This abutted the left cavernous segment and encompassed the left 5th and 6th nerves. The MRI showed no evidence of infarction. The patient was immediately commenced on oral hydrocortisone and depot injections of lantreotide. Review of symptoms two weeks later showed moderate improvement of the diplopia, however resection of the tumour will require neurosurgical intervention.
Discussion and conclusion
Pituitary adenoma should be considered as a differential in a patient presenting with a 6th cranial nerve palsy. In this case, although the cranial nerve palsy most likely resulted from direct compression by the adenoma (as suggested by the MRI), it is possible that an ischaemic neuropathy occurred secondary to impaired glucose metabolism.

DOI: 10.1530/endoabs.34.P312

P313

Optochiasmal cavernoma presenting with secondary hypogonadism: a case report

Rupa Ahluwalia[1], Muhammad Khan[1], Kumar Das[2], Ajay Sinha[3] & Jiten Vora[1]
[1]Link 7C, Department of Diabetes and Endocrinology, The Royal Liverpool and Broadgreen University Hospitals NHS Trust, Liverpool, UK; [2]Department of Neuroradiology, The Walton Centre, Liverpool, UK; [3]Department of Neurosurgery, The Walton Centre, Liverpool, UK.

Background
We report a case of a 35-year-old male presenting with pituitary dysfunction secondary to an optochiasmal cavernoma.
The gentleman was initially referred with gynaecomastia and biochemical tests consistent with secondary hypogonadism. On further questioning he also reported extremely lethargy, difficulties with weight loss and poor libido. Examination was consistent with features of hypogonadism with reduced body hair, bilateral gynaecomastia and microorchidism. Visual fields were full on direct confrontation.
Investigations
Insulin stress test noted sub-optimal responses for both GH and cortisol in the presence of adequate hypoglycaemia. GnRH test showed reduced basal levels of testosterone 0.7 nmol/l (range: 9–40 nmol/l), LH 0.7 U/l (range: 2–10 U/l) and FSH 0.6 U/l (range: 2–8 U/l) with normal LH but blunted FSH response post stimulation. TRH test showed normal TSH response with declining free T4 levels. MRI scan of pituitary showed a 1 cm hypointense mass arising from the chiasm and extending into the suprasellar cistern, suggestive of a optochiasmal cavernoma.
Management
Current treatment involves hormone replacement therapy with hydrocortisone, levothyroxine and testosterone. The patient remains under close follow-up with on-going additional radiological investigations.
Discussion
Optochiasmal cavernoma is a rare variant of cavernous hemangioma with limited cases reported in the literature. Presenting symptoms are usually related to visual field defects. Patients are prone to bleeding and stroke with sudden vision loss. MRI remains diagnostic modality of choice with differential diagnosis including craniopharyngioma and Rathke's cysts. Total surgical removal involving appropriate approach, based on size, is required for optic nerve decompression. Our case raises awareness amongst endocrinologists regarding this rare and diagnostically challenging condition that may present with a spectrum of symptoms ranging from acute vision loss to mild pituitary dysfunction.

DOI: 10.1530/endoabs.34.P313

P314

Analysis of the AIP gene promoter

Serban Radian[1,2], Peter King[1] & Márta Korbonits[1]
[1]William Harvey Research Institute, Queen Mary University of London, London, UK; [2]C. Davila University of Medicine and Pharmacy and C.I. Parhon National Inst. of Endocrinology, Bucharest, Romania.

Background
Germline mutations of the AIP tumour suppressor gene are associated with familial and sporadic pituitary adenomas, yet the tumorigenic mechanisms remain unclear. In addition, AIP protein expression in somatotroph adenomas from patients without AIP mutations correlates with clinical behaviour and

somatostatin analogues responsiveness. Understanding the regulation of AIP gene expression will help uncover its pituitary tumour-suppressor role.
Aim
To identify AIP promoter sequences and study transcription factors (TFs) regulating AIP expression.
Methods
Bioinformatics and deletion constructs analysis of human AIP promoter-pGL4.16 luciferase reporters.
Results
A −323-5 deletion construct (relative to AIP start codon) had maximal unstimulated expression in GH3 cells. A longer construct, −1314-21 (ATG-relative) had significantly lower expression, suggesting the presence of silencer sequences between −1314 and −323. The previously reported AIP promoter mutation c.−270-269CG>AA (Igreja *et al*. 2010) had a significant inhibitory effect on luciferase expression from long (−1314-21) reporter constructs. Forskolin stimulation of GH3 cells transfected with WT or −270-269CG>AA mutant AIP-promoter reporter constructs showed a significant increase of activity for the long WT construct (−1314-21), that was not observed for the mutated variant. The short WT construct (−323-5) did not respond to forskolin and the mutation had no effect on basal and forskolin-stimulated activity. Bioinformatics identified numerous TF binding sites, including two distant CREs at positions −977 and −900, but none at −270-269.
Conclusions
We have mapped the maximal activity of the AIP promoter to a small proximal region, which includes a site that is mutated in a FIPA family. The effect of this mutation on cAMP activation of the promoter seems to be indirect and requires the presence of additional distal promoter sequence to the maximally active region.
Acknowledgements
S Radian was funded by Marie Curie (PIEF-GA-2011-303006) and IESP (European Society of Endocrinology) fellowships, with additional support from the TE227/2010 project (Romanian Ministry of Education).
DOI: 10.1530/endoabs.34.P314

Reproduction

P315

Protective role of Nigerian honey on sperm indices and testis in sucrose-fed rat
Oluwakemi Oyelowo[1], Daniel Adekunbi[2] & Kayode Dada[1]
[1]University of Lagos, Lagos, Nigeria; [2]Babcock University, Ilishan-ReMO, Nigeria.

This study aimed at investigating the effect of high sucrose diet on male reproductive function and if Nigerian honey could exert a protective role. Twenty-four rats were randomly divided into four equal groups of six animals and given water (control); honey (H); high sucrose solution (30%w/v) (HSS); and both high sucrose solution (30%w/v) and honey (HSS+H). Each rat on honey received a daily dose of 10 ml honey/kg per 5 ml of distilled water. Food intake, body weight, organ weight, fasting blood glucose, LH, FSH, testosterone and sperm functions were assessed. This revealed that sperm motility ($P<0.05$) and count increased in the HSS+H and H-fed rats compared with HSS fed and control rats. Head and tail abnormalities sperm were also significantly reduced in the H fed rats ($P<0.05$). MDA level in the liver and not in the testes was significantly increased in the HSS fed rats compared with control while SOD activity was significantly increased in HSS+H rats compared with the HSS fed rats. The results indicate that sucrose feeding impact negatively on sperm function while Nigerian honey supplementation confers protective function on male reproduction.
DOI: 10.1530/endoabs.34.P315

P316

Effects of bovine somatotropin on libido, serum testosterone, hematology and certain biochemical metabolites of Sahiwal bulls
Ghulam Hussain Dilbar[1], Nazir Ahmad[1], Maqbool Ahmad[1], Ijaz Ahmad[1], Salman Waqas[1] & Muhammad Younis[2]
[1]Department of Theriogenology, University of Agriculture, Faisalabad, Pakistan; [2]Semen Production Unit, Qadirabad, Sahiwal, Pakistan.

In this study, effects of bovine somatotropin on libido, serum testosterone, hematological indices and certain serum biochemical metabolites in Sahiwal bulls were investigated. Six adult Sahiwal bulls with clinically normal reproductive tract and kept at the Semen Production Unit, Qadirabad, Sahiwal, Pakistan were used. These bulls were divided into two equal groups, i.e. treatment and control. Bulls of the treatment group were given bovine somatotropin (bST) at of 500 mg weekly, for 10 weeks, while bulls of the control group were injected with equal volume of normal saline according to the same protocol as for treatment group. Libido of these bulls was recorded fortnightly in terms of reaction time. Blood samples collected fortnightly from these bulls were analyzed for hematological parameters, while, serum samples were assayed for concentrations of testosterone and certain biochemical metabolites. Results revealed that bST treatment of Sahiwal bulls significantly decreased reaction time (5.25 ± 0.28 vs 7.50 ± 0.42 s) and increased serum testosterone concentrations (14.02 ± 0.19 vs 12.55 ± 0.44 ng/ml) compared to controls ($P<0.05$). Among hematological parameters, total leukocyte counts (10.49 ± 0.83 vs $7.91\pm0.38\times10^3/\mu l$) increased, while PCV ($36.78\pm0.64$ vs $40.78\pm1.28\%$), MCV (51.02 ± 0.47 vs 54.90 ± 0.68 fl) and MCH (16.20 ± 0.10 vs 16.86 ± 0.14 pg) decreased significantly. However, bST treatment had no effect on blood Hb, RBC counts, MCHC, ESR, differential leukocytic count and platelets count. Serum globulin (5.43 ± 0.11 vs 5.17 ± 0.04 G/dl) was higher and serum urea (12 ± 0.47 vs 15.33 ± 0.82 mg/dl) lower in bST treated bulls ($P<0.05$). However, bST had no effect on serum total proteins, albumin, glucose and SGPT activity. Thus, treatment of bulls with bST improved libido, increased testosterone, total leukocyte counts and serum concentration of globulin, and decreased PCV, MCV, MCH and serum urea.
DOI: 10.1530/endoabs.34.P316

P317

Gestational diabetes and polycystic ovary syndrome
Christiana Georgiou, Helen Mason & Gul Bano
St George's, Univeristy of London, London, UK.

Gestational diabetes mellitus (GDM) poses multiple risks to both the mother and foetus. This includes foetal macrosomia and the development of type 2 diabetes mellitus (T2DM). Women with polycystic ovary syndrome (PCOS) due to their metabolic profile are at greater risk of developing GDM and its long-term sequelae. This study aimed to investigate the prevalence and associations of GDM at St George's Hospital, with emphasis on women diagnosed with PCOS. The K2 maternal medical system was used to compile a database of 61 231 pregnancies recorded from 1 January 2002 to 26 November 12. Maternal data regarding age, ethnicity, BMI, diabetic status, PCOS status and metformin exposure was collected. Data was grouped and analysed using Microsoft Excel and conclusions were drawn from mean averages and standard error. The study found that 4.0% of the pregnant population had diagnosis of PCOS. PCOS group had an average age of 30.9 years and BMI of 26.2 kg/m2 compared with an average age of 30.2 years and BMI of 22.8 kg/m2 in non PCOS pregnancies. GDM developed in 1.9% of the pregnancies. This was more common in women with PCOS than in non PCOS women (3.7 vs 1.8%). Women from the Indian subcontinent constituted the majority of patients with GDM in both the PCOS and non PCOS groups (38.4 and 32.9%). Women with PCOS treated with metformin prior to pregnancy had a higher BMI than those PCOS women who were not treated (27.1 vs 25.6). Individuals who developed GDM despite being on metformin were exclusively from the Indian subcontinent. In conclusion, ethnicity and PCOs with higher BMI that requires treatment with metformin before pregnancy are the risk factors for GDM. Recognition of this combination of risk factors should enable clinicians to anticipate and treat GDM aggressively in this group.
DOI: 10.1530/endoabs.34.P317

P318

Plant molecules quercetin and resveratrol can affect ovarian cells and invert FSH action
Aneta Stochmalova[1,2], Attila Kadasi[2,3], Richard Alexa[1,2] & Alexander Sirotkin[1,2]
[1]Department of Zoology and Anthropology, Constantine the Philosopher University, Nitra, Slovakia; [2]Institute for Genetics and Reproduction of Farm Animals, Animal Production Research Centre Nitra, Luzianky near Nitra, Slovakia; [3]Department of Animal Physiology, Faculty of Biotechnology and Food Science, Nitra, Slovakia.

Quercetin and resveratrol, powerful antioxidants and blockers of intracellular mTOR signalling system, are widely distributed in edible plants. Their action on ovarian cell functions and the interrelationships with gonadotropin are studied insufficiently. We examined the effect of FSH, quercetin, resveratrol and their

combinations on proliferation and apoptosis of cultured porcine ovarian granulosa cells. Accumulation of marker of proliferation (PCNA) and marker of apoptosis (bax) was determined by immunocytochemistry. It was observed that FSH increased the expresion of PCNA and decreased the expresion of bax in cultured ovarian cells. Either quercetin and resveratrol, when added alone, decreased the occurence of proliferation marker and increased the expression of apoptosis marker. FSH in combination with either quercetin and resveratrol decreased the occurence of PCNA and increased the expression bax. Therefore, these plant extracts were able to prevent and even invert FSH action. Our observations suggest, that FSH can up-regulate, and plant molecules quercetin and resveratrol can down-regulate ovarian cell functions, and that the intake of these plant molecules can inhibit and even invert FSH action on ovarian cells. Furthermore, it is possible, that FSH action on ovarian cell functions can be mediated via mTOR intracellular signalling system.

Key Words: FSH, quercetin, resveratrol, mTOR, proliferation, apoptosis, porcine granulosa cells

DOI: 10.1530/endoabs.34.P318

P319

Fetal glucocorticoid overexposure impacts on germline epigenetic reprogramming in the rat

Catherine M Rose, Sander van den Driesche, Ashley K Boyle, Thomas Chambers, Richard M Sharpe, Richard R Meehan & Amanda J Drake
University of Edinburgh, Edinburgh, UK.

Background
Fetal glucocorticoid overexposure is associated with low birthweight and increased cardiovascular disease risk in the offspring. Such 'programmed effects' can be transmitted across generations through both male and female lines. Disruption of a germline epigenetic reprogramming pathway, characterised by genome-wide erasure and subsequent re-establishment of DNA methylation, may underpin the intergenerational transmission of programmed effects. We used a rat model to explore the hypothesis that glucocorticoid overexposure affects DNA re-methylation in germ cells in late gestation.
Methods
Pregnant female Wistar rats were treated with dexamethasone (Dex:100 μg/kg per day) or vehicle from E15.5 onwards. Testes were collected between E18.5 and E21.5, and immunofluorescence used to localise the glucocorticoid receptor (GR), 5-methylcytosine (5 mC) and the DNA methyltransferases DNMT3a, 3b and 3L. To explore whether Dex affected testis maturation we assayed for the presence of the double-sex and mab-3 related transcription factor 1 (DMRT1), an important transcription factor in early testicular differentiation, which is undetectable in germ cells after E19.5.
Results
GR was detectable in germ cells throughout the time course. Dex treatment was associated with a greater number of 5 mC positive germ cells at E19.5 but this difference was no longer apparent at E20.5. DNMT3a, 3b and 3L were detected in germ cells throughout the re-methylation phase in both treatment and control groups. DMRT1 was present in germ cells at E18.5 and E19.5, and absent by E20.5 in both groups, suggesting that the shift in the timing of re-methylation may not correspond to premature maturation across the entire testis.
Conclusions
Our observations suggest that Dex exposure is associated with earlier germ cell re-methylation in the absence of global changes in the DNMTs. The mechanism(s) mediating this change remain to be elucidated.

DOI: 10.1530/endoabs.34.P319

P320

Post-endocytic sorting of the LH receptor is mediated by a novel APPL1 dependent mechanism

Silvia Sposini, Frederic Jean-Alphonse, Ashley Newcombe & Aylin Hanyaloglu
Imperial College, London, UK.

LH receptor (LHR) is a G protein-coupled receptor (GPCR) that plays key roles in reproduction and pregnancy. Endocytic trafficking and sorting of GPCRs to diverse cellular fates represent a key mechanism in defining cellular responses by controlling both the temporal and spatial parameters of cellular signalling. How different processes within the endocytic system are coordinated, however, needs to be mechanistically dissected in more detail. We have recently demonstrated that the interaction of human LHR with the PDZ domain protein GIPC targets the receptor to pre-early endosomes (pre-EEs), specific compartments upstream of the classic early sorting endosome. Targeting of LHR to pre-EEs by GIPC is necessary for receptor sorting to the recycling pathway and for a sustained MAPK signalling profile from pre-EEs. Pre-EEs were also positive for the adaptor protein APPL1 (adaptor protein containing pleckstrin homology PH domain, PTB domain and leucine zipper motif 1). Although APPL1 was not required for LHR endosomal localization, it was essential for receptor recycling, a function not previously ascribed for APPL1 for any membrane cargo. Therefore, this study aimed to characterize the molecular mechanisms mediating LHR recycling. LHR trafficking was visualised via total internal reflection fluorescence (TIRF) microscopy. APPL1 mutants, each lacking one or more C-terminal domains, were tested for their ability to rescue LHR impaired recycling in cells stably expressing shRNA APPL1. Interestingly, mutation of a PKA phoshorylation site, known to interact with the phosphatase OCRL, could not rescue LHR recycling in APPL1 depleted cells. Furthermore, inhibition of PKA dramatically inhibited LHR recycling suggesting a role for LHR signal activation in regulating APPL1 interactions and its sorting from pre-EEs. Overall these findings reveal an unprecedented sorting mechanism for signalling receptors, mediated by APPL1. Diversity in sorting mechanisms raises the possibility that post-endocytic sorting could be programmed for an individual receptor at multiple levels.

DOI: 10.1530/endoabs.34.P320

P321

Fertility and pregnancy outcomes for patients with polycystic ovary syndrome in the UK: a retrospective observational study

Christopher Morgan[1], Sara Jenkins-Jones[2] & Aled Rees[3]
[1]Institute of Primary Care and Public Health, Cardiff University, Cardiff, UK; [2]Pharmatelligence, Cardiff, UK; [3]Institute of Molecular and Experimental Medicine, Cardiff University, Cardiff, UK.

Background
Polycystic ovary syndrome (PCOS) is characterised by anovulation and insulin resistance but the effects on reproductive outcomes are unclear.
Objectives
To determine the impact of PCOS upon fertility, pregnancy complications and delivery method.
Methods
Data were extracted from the Clinical Practice Research Datalink. Patients with a diagnosis of PCOS (2000–2012) were matched to controls (1:2) by age (±1 year), BMI (± 3 units) and primary care practice. Date of PCOS diagnosis defined index date. Time between index date and first consultation for infertility was calculated and prevalence of infertility compared. Standardised fertility ratios (SFR) before/after diagnosis were calculated. Rates of pre-eclampsia, gestational diabetes (GDM), preterm delivery, miscarriage and delivery method were also compared.
Results
9068 women with PCOS matched study criteria. Prior to index date, 1529 (16.9%) PCOS women consulted for fertility compared with 800 (4.4%) controls: a crude rate ratio (RR) of 4.69 (95% CI 4.30–5.11). Respective figures post index date were 796 (8.8%) vs 496 (2.7%): a crude RR of 3.59 (3.21–4.02). Prior to index date the SFR for patients with PCOS was 0.83 (0.80–0.86), following index date it was 1.17 (1.13–1.21). Risk of pre-eclampsia was 1.14 (1.00–1.30) and GDM was 1.30 (1.13–1.49). Of PCOS births, 27.8% were by Caesarean section compared with 23.8% of non-PCOS. In logistic regression the odds ratio of Caesarean delivery for PCOS was 1.13 (1.05–1.20) after adjustment for pre-eclampsia (2.62 (2.25–3.05)) and GDM (2.53 (2.14–3.00)). Mean length of stay for delivery was greater for PCOS (3.8 vs 3.5 days, $P=0.002$). Risk of miscarriage (RR 1.53 (1.43–1.64)) and premature delivery (RR 1.29 (1.15–1.45)) were also increased.
Conclusions
Infertility is associated with PCOS but fertility rates improve significantly following treatment. GDM, pre-eclampsia, miscarriage and pre-term delivery were more prevalent for women with PCOS and births were more likely to be by Caesarean delivery.

DOI: 10.1530/endoabs.34.P321

P322

In the mouse ovary AMH expression is independent of androgen physiology

Marie Lebbe[1] & Teresa Woodruff[2]
[1]University of Birmingham, Birmingham, UK; [2]Northwestern University, Chicago, USA.

Background
Anti-Müllerian hormone (AMH) is a key regulator of preantral follicle development. In human polycystic ovarian syndrome (PCOS) hyperandrogenism drives antral follicle excess, and is associated with elevated AMH levels. It is currently unknown if androgens regulate AMH secretion.
Objective and hypothesis
To provide insights into the regulation of AMH action, we hypothesized that dihydrotestosterone (DHT), the most potent androgen, stimulates AMH production in preantral follicles.
Methods
We cultured day 5 CD1 mouse ovaries, containing mostly primordial and preantral follicles, with DHT supplementation vs control. After 4 days of culture, medium was collected for protein analysis and ovaries for RNA analysis and histology. We performed follicle counting, qRT-PCR for mRNA levels of AMH, AMH measurements by ELISA and AMH-immunofluorescence.
Results
In the employed culture model, using DHT at physiological concentrations of 1×10^{-7} and 1×10^{-8} M, DHT does not increase the preantral follicle pool, neither upregulate mRNA levels for AMH. Protein levels for AMH were similar in control and treatment conditions, and no dose-dependant DHT effect was observed.
Conclusion
In mice, AMH expression appears not to be regulated by androgens. In contrast to women, mice lack adrenal androgen synthesis and are poly-ovulators. These data suggest that the mouse ovarian model might be inadequate to study the role of androgens in preantral follicle development, and generate data that are translatable to the human.

DOI: 10.1530/endoabs.34.P322

P323

Interaction of androgen with IGF signalling in preantral follicle development in the mouse ovary

Hina Jamall, Mhairi Laird, Kate Hardy & Stephen Franks
Imperial College London, London, UK.

Polycystic ovary syndrome (PCOS) is the commonest endocrine disorder in women of reproductive age. It is characterised by excessive ovarian androgen production which, in turn, has been implicated in the aetiology of aberrant follicular development. We have previously reported that prenatal exposure to androgens activates follicle growth[1]. It has been suggested that androgens may interact with the IGFs to promote the activation and growth of follicles. The aim of this study was to investigate the effects of androgen on the type 1 IGF receptor (IGFR1) and the transcription factor, Forkhead box (Foxo)3a (a downstream target of IGF signalling), using cultured whole ovaries from neonatal mice.
Whole ovaries were collected from neonatal C57BL/6 mice at PND 4 and cultured, in the presence or absence of dihydrostestosterone (DHT – a non-aromatisable androgen), for 7 days. At the end of culture ovaries were fixed for immunohistochemical localisation of IGFR1 or Foxo3a. Image analysis was carried out to assess the proportion of primordial, and developing follicles, the abundance of IGFR1 and the subcellular localisation of Foxo3a.
Treatment with DHT (10 nM) decreased the proportion of primordial follicles and increased the proportion of multi-layered follicles compared to control ($P<0.001$). Foxo3a and IGFR1 were observed in the oocyte and granulosa layer, respectively, of every follicle analysed. Treatment with androgen caused an increase in the proportion of cytoplasmic expression and a decrease in the proportion of nuclear Foxo3a in primordial, transitional and multilayered follicles compared to control ($P<0.001$ and $P<0.05$, respectively). Furthermore, the addition of DHT resulted in increased IGFR1 protein expression in transitional and multi-layered follicles ($P<0.0001$). In conclusion, this study provides strong evidence that androgen dependent dysregulation of growth of preantral follicles is exerted through interactions with Foxo3a and IGFR1, key factors known to stimulate follicle activation and growth. This study is supported by MRC programme grant G0802782.
1. Forsdike RA, *et al*. *J Endocrinol* 2007 **192** 421.

DOI: 10.1530/endoabs.34.P323

P324

Regulation of implantation by interaction between the IGF receptor (IGF1R) and miR-145

Miranda Lees[1,2], Youn-Jung Kang[1,2], Karen Forbes[1,2] & John Aplin[1,2]
[1]Maternal and Fetal Health Research Centre, University of Manchester, Manchester, UK; [2]St Mary's Hospital, Central Manchester University Hospitals NHS Foundation Trust, Manchester, UK.

Successful implantation requires the synchronisation of viable embryonic development with endometrial receptivity. The mechanisms allowing for the initiation of crosstalk remain elusive, however recent studies have revealed alterations in endometrial microRNAs (miRs) in women suffering repeated implantation failure (RIF). We hypothesised that the IGF1 receptor (IGF1R) is involved in implantation, and that miR145, which is elevated in RIF endometrium and predicted to target IGF1R, influences epithelial receptivity. We have used an *in vitro* model to study the effect of miR145 on IGF1R expression and early implantation events.
Overexpression of miR-145 in Ishikawa cells reduced the level of IGF1R protein (western blotting), but not mRNA (RT-qPCR). 3′UTR luciferase reporter assays demonstrated that miR-145 directly regulates IGF1R mRNA.
Mouse embryos co-cultured with Ishikawa cells were observed to attach over a period of 24 h. Stability of attachment was impaired upon overexpression of miR-145 or specific reduction of IGF1R (siRNA).
To demonstrate functional IGF1R at the cell surface, we transferred embryo-sized beads coated with IGF1 to cell monolayers and observed attachment. Knockdown of IGF1R decreased the interaction, demonstrating receptor specificity. Similarly, impaired attachment was observed after miR-145 overexpression.
To determine if IGF1R mediates the functional effect of miR-145 overexpression on attachment, we prevented the interaction between miR-145 and IGF1R using miR-target protectors, and reversed the effect of miR-145 overexpression.
The data show that miR-145 influences embryo attachment by regulating IGF1R in endometrial epithelial cells, suggesting the two have opposing effects in regulating implantation.

DOI: 10.1530/endoabs.34.P324

P325

Renal anomalies in Kallmann syndrome, an uncommon association

Preeti Chiran
St Helens and Knowsley NHS Trust, Prescot, UK.

We present the case of an 18-year-old boy with phimosis who was referred by the urology team as he had low levels of testosterone. Clinical evaluation revealed that he had normal pubertal growth spurt and was 183 cm tall. His sense of smell was impaired and voice was high pitched. Examination revealed no facial hair, scanty pubic and axillary hair. He had bilateral gynaecomastia, phimosis and micropenis (1.0 cm). The testes were small and soft in consistency (right 2.0 and left 1.0 ml).
Investigations revealed testosterone 0.7 nmol/l (nr 8.0 – 34.0), LH 0.2 IU/l (1.0–7.0) and FSH 0.8 IU/l (1.0–6.0). Ferritin, prolactin, oestradiol, IGF1, thyroid function tests and Short Synacthen test were normal. MRI pituitary and karyotype (46XY) were normal. He underwent surgery to correct phimosis and further investigations revealed duplex right renal collecting system and single joint ureter. A diagnosis of anosmic hypogonadotrophic hypogonadism (Kallmann syndrome) with renal anomalies was made.
He was initiated on testosterone gel and later switched to testosterone undecanoate, 1000 mg 12 weekly. Treatment resulted in growth of facial and body hair and his voice broke. There was no change in the size of external genitalia. This case highlights the importance of considering Kallmann syndrome in the presence of renal tract anomalies including phimosis.
Conclusion
The case report highlights the importance of considering Kallmann syndrome in young men with renal anomalies.

DOI: 10.1530/endoabs.34.P325

P326

miR-145 is associated with placental growth in mice

Lisa Jones, Jayne Charnock & Karen Forbes
Institute of Human Development, Maternal and Fetal Health Research Centre, University of Manchester, Manchester, UK.

The IGF axis is imperative for normal placental development and function. In the human placenta, IGF1R regulates the actions of IGFs and consequently placental growth. Some microRNAs (miRs) are also known to regulate human placental growth. miR-145 is known to exert its effects on human placental growth by targeting IGF1R. Whilst the expression of some components within the IGF axis are similar between mice and humans, murine expression and function of IGF regulatory miRs has yet to be established. We therefore examined the expression of miR-145 and its target IGF1R, and their relationship to growth in the mouse placenta. Placentas from C57 mice were harvested at E12.5, E15.5 and E18.5. At all gestational time-points miR-145 and IGF1R mRNA was assessed by RT-qPCR and normalised to 5srRNA and 18srRNA, respectively. Immunohistochemistry was used to assess IGF1R protein expression. Placental proliferation was assessed by Ki67 immunohistochemistry and subsequent *in silico* analysis. RT-qPCR demonstrated that miR-145 is expressed in the mouse placenta. miR-145 levels increased with gestation ($P<0.05$), whilst placental proliferation significantly decreased over gestation ($P<0.05$). There was no change in placental IGF1R mRNA throughout pregnancy, however, IGF1R protein expression decreased towards term; this discrepancy between mRNA and protein expression is consistent with the known actions of miRs on protein translation. Whilst further work is required to confirm the direct effect of miR-145 in the mouse placenta, this inverse correlation between miR-145 and proliferation/IGF1R is consistent with studies in the human placenta. This suggests that miR-145 is also likely to regulate placental growth in mice by modulating IGF1R expression. Ongoing work will investigate the effect of administering miR-145 regulatory drugs *in vivo* in mice. If, as expected, effects on placental growth are observed, manipulation of placental miR-145 levels may prove useful to improve pregnancy outcomes associated with aberrant placental development.

DOI: 10.1530/endoabs.34.P326

P327

Glial cells missing 1 transactivates the equine chorionic gonadotrophin beta promoter

Victoria Cabrera-Sharp[1], Jordan Read[1], Phoebe Kitscha[1], Amelie Geddis[1], Judith Cartwright[2] & Amanda de Mestre[1]
[1]Royal Veterinary College, London, UK; [2]St Georges, University of London, London, UK.

Placental chorionic gonadotrophin (CG) hormone secretion, critical for maintenance of early pregnancy, is dependent on differentiation of specialised CG-secreting binucleate trophoblast (horse) and syncytiotrophoblast (human). The most abundant genes expressed by binucleate cells are the α and β subunits of equine CG. We recently showed the transcription factor glial cells missing 1 (GCM1) is rapidly induced during differentiation of binucleate trophoblast and mRNA expression precedes and closely correlates with the expression of CGβ mRNA *in vivo*. Here, we confirmed by western blotting that GCM1 protein is predominantly expressed in the chorionic girdle of the equine conceptus and becomes up-regulated correlating with the induction of CGβ mRNA. Bioinformatic analysis of a 3000-bp fragment of equine CGβ promoter revealed five exact match consensus sites available for GCM1 binding; two located within 200 bp immediately upstream of the transcription start site. A 335 bp fragment, containing these two GCM1 sites, was cloned into pGL3 firefly luciferase vector (Promega) and sequenced. BeWo choriocarcinoma cells were transiently transfected with pGL3-335 or pGL3 basic (as a control) using, Lipofectamine 2000 (Invitrogen), in addition to pRL-SV40 Renilla luciferase vector (Promega) (transfection control). Promoter activity was measured using a Dual-Glo Luciferase Kit (Promega). BeWo cells transfected with pGL3-335 demonstrated a 37-fold increase in luciferase activity relative to control transfected cells at 24 h post transfection. Furthermore, co-transfection of a GCM1 expression vector (pEGFP-GCM1) in increasing amounts with pGL3-335 into BeWo cells resulted in a twofold increase in luciferase activity compared to control transfected cells (pGL3-335, pEGFPN1). In conclusion, these results support our hypothesis that GCM1 regulates equine CG expression, exerting its function via binding to the CGβ promoter. Ongoing studies are investigating the ability of GCM1 to bind to the CGβ promoter *in vivo*.

DOI: 10.1530/endoabs.34.P327

P328

Hypoxia induces terminal differentiation of primary trophoblast cells *in vitro*

Victoria Cabrera-Sharp, Jordan Read, Abir Mukherjee & Amanda de Mestre
Royal Veterinary College, London, UK.

Equine chorionic gonadotrophin (eCG) is not only essential for the maintenance of early equine pregnancy but is also used therapeutically for synchronisation of farm and laboratory animals. Currently it is extracted from serum of farmed pregnant mares. We have recently reported that under normoxic conditions, BMP4 treatment of equine trophoblast resulted in a dose, and developmental stage-dependent increase in total number of differentiated binucleate cells that secrete eCG. Owing to the late implantation of the equine conceptus, we hypothesise that the binucleate trophoblast cells of the equine placenta are induced to differentiate in a low oxygen environment. The objective of this study was to observe the functional response of BMP4 treated trophoblast cells to reduced oxygen conditions. Normal conceptuses were obtained by non-surgical uterine lavage at days 30–31 of pregnancy. Pure populations of day 30 and 31 primary chorionic girdle trophoblast (corresponding to the initiation of terminal differentiation *in vivo*) were cultured in the presence/absence of 100 ng/ml human BMP4 in either 20% oxygen or in a modified gas composition of 5%CO_2, 5%O_2 and 90% N. Differentiation status was determined through quantification of nuclei number of each cell in five fields of view from each sample. We report, under normoxic conditions, at least a twofold increase in total number of differentiated binucleate cells in response to 100 ng/ml BMP4 in both day 30 and day 31 cells taken straight from the conceptus and cells passaged up to three times. Interestingly, we observed a fivefold increase in total number of binucleate cells cultured in the absence of BMP4 at 5% O_2 compared to 20% O_2 at day 30 of gestation. Furthermore, BMP4 had no additional effect on differentiation when cells were cultured at 5% O_2. In conclusion, these results show that hypoxic conditions alone can induce trophoblast differentiation *in vitro*.

DOI: 10.1530/endoabs.34.P328

P329

A rare cause of infertility

Ahmed Imran Siddiqi & Ravi Menon
University College London, London, UK.

Case history
A 29-year-old man presented with infertility. He also complained of difficulty in passing urine. He also complained of low libido and ejaculate was also noted to be watery. He complained of occasional difficulty in maintaining erections. He denied history of trauma, radiation exposure or use of illicit drugs. He had normal pubertal development and secondary sexual features. He had undergone a surgery about 11 years ago in Nigeria for hypospadias. Hypospadias was identified at the time of his birth. He then moved to UK and started experiencing these symptoms about 2 years ago. The testicular volume was about 7 mls bilaterally with curved small penis with chordae and eccentric meatus with fistula. Normal secondary sexual features.
Investigations
Hormone profile: FSH 25 IU/l (1.5–12.4); LH 14.9 IU/l (1.7–8.6), testosterone 8.3 nmol/l (7.6–31.4). Semen analysis: azoospermia. The karyotype analysis: 46, XX; pattern of X chromosome normal. No SRY (sex-determining region on the Y chromosome) of Y chromosome was detected with microdeletion PCR array.
MRI abdomen and pelvis: Miillerian duct remnants in the midline posteriorly. Small gonads in scrotum, small prostate identified. However, no uterus or cervix found. No other gonadal tissue identified.
Bone mineral density: mild osteopenia.
Treatment
Given the partial gonadal failure and his desire to improve erections and libido, he was started on testosterone replacement. He had corrective surgery for the urethral meatus and penile curvature. His case is still being discussed for micro TESE although, limited literature available for this chromosomal abnormality is not very supportive of this approach.
Conclusion
We present here a case with rare chromosomal abnormality who presented with infertility. 46 XX with SRY negative is rare cause of infertility.
Learning points
Karyotyping should be considered in appropriate adult patients which could give answer to the presenting problem.

DOI: 10.1530/endoabs.34.P329

P330

Transforming growth factor-β superfamily signalling and its role in the pathogenesis of heavy menstrual bleeding

Jacqueline Maybin, Lyndsey Boswell, Colin Duncan & Hilary Critchley
MRC Centre for Reproductive Health, Edinburgh, UK.

Introduction
The human endometrium has a remarkable capacity for repeated repair following the inflammation of menstruation. This occurs without scarring or loss of function but mechanisms involved remain undefined. Aberrations in endometrial repair may lead to pathology such as heavy menstrual bleeding (HMB). The transforming growth factor-β superfamily has been implicated in efficient wound repair and has a potential role in menstrual repair. Downstream of TGF-β, phosphorylation of Smad2/3 initiates binding with Smad4 to regulate target genes.
Hypotheses
Women with HMB have i) delayed endometrial repair and ii) aberrant endometrial TGF-β signalling compared to women with normal loss (NMB).
Methods/results
Endometrial biopsies ($n=44$) were collected with ethical approval and consent from women with no structural abnormalities. Stage classification was based on day of cycle, histology and serum hormone levels at time of biopsy. Objective measurement of menstrual blood loss was performed using the alkaline–haematin method to classify women as having HMB >80 ml or NMB <80 ml. Menstrual pictograms were completed simultaneously. Of note, women with HMB bled for 6 days on average, compared to 4 days for women with NMB ($P<0.01$). Gene expression between these two groups was compared using RT-PCR. Menstrual phase endometrium from women with HMB had significantly less SMAD3 mRNA expression than those from women with NMB ($P<0.05$). There were no significant differences in SMAD3 expression at any other phase of the menstrual cycle. Phosphorylated SMAD2/3 protein was identified by immunohistochemistry in menstrual phase endometrium, present in a nuclear location in glandular epithelial cells. Minimal staining was seen in proliferative and early secretory tissue.
Conclusions
The prolonged bleeding identified in women with HMB is consistent with defective endometrial repair. The presence of active SMAD2/3 protein at menstruation and decreased SMAD3 expression at this time in women with HMB implicates TGF-β signalling in the pathogenesis of this common disorder.

DOI: 10.1530/endoabs.34.P330

P331

Effect of endometrial thickness on pregnancy outcome after ICSI

Rehana Rehman[1], Syeda Sadia Fatima[2], Taseer Khan[3], Mehwish Hussain[4] & Rakhshaan Khan[5]
[1]Bahria University, Karachi, Sindh, Pakistan; [2]Aga Khan University, Karachi, Sindh, Pakistan; [3]Karachi University, Karachi, Sindh, Pakistan; [4]Dow University, Karachi, Sindh, Pakistan; [5]Bahria University, Karachi, Sindh, Pakistan.

Objective
To study association of endometrial thickness measured on ovulation induction (OI) day with cycle outcome after ICSI.
Subjects and methods
It was a quasi experimental design carried out in an infertility clinic from July 2011 till June 2012. The treatment comprised of down regulation of ovaries, controlled ovarian stimulation, oocyte pick up, IVF, embryo (blastocysts) transfer followed by confirmation of pregnancy with beta hCG more than 25 mIU/ml. On OI, before human chorionic gonadotrophin (hCG) injection, endometrial thickness was measured by trans vaginal scan and serum; estradiol (E_2), progesterone (P) and interleukin 1β (IL1β) were measured by Enzyme Linked Immuno Sorbent Assay. The cut off value of endometrial thickness for pregnancy was evaluated by receiver operating curve (ROC) and characteristics of stratified groups were compared on the basis of Student's *t*-test.
Results
Results of 282 couples on the basis of ROC stratified patients into two groups; Group A; non pregnant = 159) with a cut off thickness of <8 and group B 123 pregnant patients with ≥ 8 mm thickness. A high E_2, IL1β and less *P* on OI ($P<0001$) was found in pregnant patients.
Conclusion
The endometrial thickness of 8 mm was associated with a positive pregnancy outcome after ICSI. These patients showed better oocyte parameters, number of embryos, raised peak E_2 and IL1β and less *P* levels on OI day.

Key Words
ICSI, ovulation induction, serum estradiol, serum progesterone, serum interleukin 1β, endometrial thickness, implantation rate

DOI: 10.1530/endoabs.34.P331

P332

Regulation of the LH/CG receptor signalling in human endometrium

Camilla West[1], Aylin Hanyaloglu[1], Jan Brosens[2], Stuart Lavery[1] & Nick Panay[1]
[1]Imperial College London, London, UK; [2]University of Warwick, Warwick, UK.

It is well established that the interaction between an implanting embryo and the endometrium is essential for successful implantation and there is a variety of locally secreted factors within the uterine environment which underlies this maternal–fetal cross-talk. Human chorionic gonadotrophin (hCG) is a glycoprotein hormone secreted by the embryo and is essential during early stages of pregnancy to maintain progesterone production from the corpus luteum. Its receptor (LH/CGR), a G-protein coupled receptor (GPCR), is known to be expressed in the endometrium but the role of hCG and the underlying signalling mechanisms here are largely unknown. We show that the LH/CGR in human endometrial stromal cells (HESCs) acts via a Gαi pathway instead of the classical Gαs pathway and that signalling via this receptor may modulate important decidual-specific genes. We also observe a change in trafficking of the LH/CGR between the two cell–states of HESCs with control cells showing constitutive trafficking which is inhibited upon differentiation/decidualisation. However, the receptor is not visibly internalised following stimulation with hCG in undifferentiated or differentiated cells. This is specific to the LH/CGR as the same 'switch' in receptor trafficking is not observed with another classical GPCR, the β2-adrenergic receptor. We also show that a region of the C-terminal tail of the receptor dictates its endocytic trafficking, with more receptor localising to EEA1 positive early endosome compartments when this sequence is deleted. Furthermore, knockdown of the PDZ binding protein GIPC alters the endocytic trafficking of the WT receptor, re-routing it to larger endosomal compartments. These observations are consistent with LH/CGR trafficking in HEK293 cells, which primarily trafficks to an endosomal compartment upstream of early endosomes (pre-early endosomes). Dynamic alterations in the trafficking of the receptor in the endometrium may be an important regulatory mechanism in hCG actions on the endometrium during the crucial period of implantation.

DOI: 10.1530/endoabs.34.P332

P333

Steroid hormones regulate cyclical expression of osteopontin and CD44 in the ovine endometrium

Tina Tremaine, Mohammed Khalid, Claire Wathes & Ali Fouladi-Nashta
The Royal Veterinary College, London, UK.

Successful embryo implantation requires changes in endometrial gene expression of biomarkers which contribute to uterine receptivity to facilitate blastocyst attachment. Osteopontin (OPN), expressed at the maternal–foetal interface may facilitate implantation in processes, such as cell adhesion, migration and angiogenesis which require OPN signalling via CD44 and integrin receptors. As coordinate expression of OPN and receptors is shown to be cycle-dependent and up-regulated in human, mice and ovine endometrium at implantation, it is logical to conclude regulation of OPN may require ovarian steroids and embryonic signals. Aims of this study were to analyse steroid regulation and cyclical expression of OPN and receptors in ovine uterus using both *in vitro* and *in vivo* endometrial models.
Uteri were obtained from sheep sacrificed at follicular, luteal cycle phases, early gestation (d.17 and 35) and ovariectomised animals intramuscularly treated with E_2 (3 μg/ml) ± P_4 (12.5 mg/ml). OPN mRNA expression was determined by qRT-PCR and protein levels of OPN and receptors were detected by SDS–PAGE-western immunoblotting and immunohistochemistry. Populations of luminal epithelia and stromal cells were isolated from luteal phase endometrium, and grown in the presence or absence of $E_2 \pm P_4$, to analyse steroid hormone effects on OPN expression in isolated cell types.
Analysis of OPN and receptors in cyclical and early pregnant ewes confirmed up-regulation of OPN in luteal phase endometrium and early implantation stages. Western-blot analysis revealed the presence of 70-, 32- and 15-kDa OPN isoforms in endometrium which may result from progesterone regulation of posttranslational modifications of the native 70-kDa form. In addition, progesterone

(10 ng/ml) significantly increased OPN expression in stromal cells, the effect of which was blocked by progesterone receptor antagonist, but P_4-regulated OPN expression was not shown in epithelial cells. These results demonstrate the coordinate expression of OPN and receptors regulated by progesterone coincide with the timing of implantation.

DOI: 10.1530/endoabs.34.P333

P334

Oestrogen implant improves symptoms of hypogonadism and lipid profile in transwomen

Ye Kyaw, Maricel Espina & Leighton Seal
Thomas Addison Unit, St George's Hospital, Tooting, London, UK.

Background
Oestrogen replacement therapy is essential in transwomen for their well-being. Various forms of oestrogen therapy are available. Individual responses to such replacement therapy may vary. Hormonal implant is a reliable form of hormone replacement in postmenopausal women. However, their efficacy and safety in transwomen have not been thoroughly examined yet.
Methods
14 transwomen had 100 mg of oestradiol implant inserted subcutaneously in the anterior abdominal wall. All women had previously been on other forms of oestrogen replacement. Patients were interviewed for symptoms of low sex hormones, their satisfaction on treatment and presence of complications before their first implant and in a subsequent visit. Blood tests were done before the 1st implant was inserted and on a subsequent visit before the implant was inserted. They were also asked to grade their energy level, general drive and libido from a scale of 0–10. Pre and post implant data were analysed by using paired *t*-tests.
Findings
Total cholesterol level decreased from 5.1 ± 0.7 to 4.7 ± 0.8 mmol/l ($P = 0.046$) and triglyceride level also reduced from 1.6 ± 0.2 to 1.2 ± 0.5 mmol/l ($P = 0.05$). Energy level (4.5 ± 2.2 vs 7.2 ± 2.1, $P = 0.000$), general drive (5.1 ± 2.2 vs 7.8 ± 1.3, $P = 0.000$) and libido (3.0 ± 2.4 vs 5.9 ± 2.8, $P = 0.002$) improve post implant in comparison with pre-implant symptoms. No significant change in liver enzymes was noted. Estradiol level also increased significantly (294 ± 246 vs 573 ± 227 pmol/l, $P = 0.000$). Compared to previous oestrogen replacement in other forms, 64.3% of the patients said implant therapy was better whereas 26% said it was the same. 1 patient (7%) said it was worse than previous treatment. There no complications in all 14 transwomen studied.
Conclusion
This study is the first study to look into the use of oestrogen implant in transwomen. It not only improves symptoms but also improves lipid profile in transwomen. Therefore, oestrogen implant treatment is a safe and effective therapy with high patient satisfaction. It should be considered as a form of replacement in transwomen who do not respond to other forms of oestrogen therapy.

DOI: 10.1530/endoabs.34.P334

P335

Testosterone implant therapy: efficacy and safety in transmen and native men with hypogondism

Ye Kyaw[1], Maricel Espina[1,2] & Leighton Seal[1]
[1]Thomas Addison Unit, St George's Hospital, Tooting, London, UK; [2]Gender Identity Clinic, Charing Cross Hospital, London, UK.

Background
Various forms of testosterone replacement therapy has been used in replacement therapy for transmen and men with hypogonadism. However, efficacy and safety of testosterone implant has not been studied yet.
Methods
11 patietns (five transmen and six men with hypogonadism) had 800–1000 mg of testosterone implant inserted subcutaneously in the anterior abdominal wall. All subjects had previously been on other forms of testosterone replacement. Patients were interviewed for symptoms of low sex hormones, their satisfaction on treatment and presence of complications before their first implant and in a subsequent visit. Blood tests were done before the 1st implant was inserted and on a subsequent visit before the implant was inserted. They were also asked to grade their energy level, general drive and libido from a scale of 0–10. Pre-and post-implant data were analysed by using paired *t*-tests.
Findings
In a scale of 0–10, patient's energy level (4.7 ± 2.2 vs 7.1 ± 2.0, $P = 0.026$), general drive (5.1 ± 2.1 vs 7.6 ± 1.2, $P = 0.003$) and libido (3.0 ± 2.6 vs 7.2 ± 2.4, $P = 0.002$) improve post-implant in comparison with pre-implant symptoms. No significant change in liver enzymes or lipid profile were noted. Haemoglobin (15.1 ± 1.6 vs. 15.6 ± 1.8 g/dl, $P = 0.15$) and haematocrit (0.45 ± 0.05 vs. 0.47 ± 0.05, $P = 0.07$) were not significantly different pre- and post- implant. Serum testosterone level did not change significantly pre- and post-implant (9.3 ± 9.0 vs. 10.7 ± 3.9 mmol/l, $P = 0.64$). Compared to previous testosterone replacement in other forms, 73% of the patients said implant therapy was better whereas 27% said it was the same. There was no complications in all 11 patients studied.
Conclusion
Testosterone implant improves symptoms of hypogonadism. None of the subjects had any complications or side effects such as polycythaemia. Therefore, testosterone implant treatment is a safe and effective therapy for symptoms improvement in transmen and men with hypogonadism. It may be considered as a form of replacement in selected patients who do not respond to other form of testosterone replacement.

DOI: 10.1530/endoabs.34.P335

P336

Elevation of HRPE773 (ZG16B) expression in amnion at term and in human ectocervical cell lines treated with inflammatory mediators is consistent with a function in innate immunity

Rachel Rachel Hayhurst[1], Nanette Hibbert[1], Lorraine Frew[1], Sarah Stock[1], Simon Riley[1] & Steven Morley[2]
[1]MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK; [2]Division of Health Sciences, University of Edinburgh, Edinburgh, UK.

Introduction
Labour is an inflammatory process involving the innate immune system, premature activation of which, for example by infection, can lead to preterm birth before 37 weeks of gestation with profound pathophysiological consequences for the offspring. The putative antimicrobial protein HRPE773 (ZG16B) expressed in human female reproductive secretory epithelia, has been shown previously to be elevated in human endometrium during the menstrual early secretory phase and in uterine decidua following miscarriage. We therefore hypothesized that HRPE773 expression might be regulated by inflammatory stimuli in female reproductive tissues.
Methods
Human amnion, chorio-decidua, placenta, myometrium and cervix collected with informed consent from donors undergoing natural labour at 40+ weeks gestation or elective caesarean section between weeks 39 and 43 gestation, were obtained through the Edinburgh Reproductive Tissue Bio Bank. QRT-PCR using the $2-\Delta\Delta Ct$ method with 18SRNA as internal standard and immunohistochemistry were used respectively to determine HRPE773 mRNA levels and protein localization. HRPE773 expression was also measured in RNA from ectocervical (ECT1/E6E7) and endocervical (END1/E6E7) cell lines treated with IL1β or LPS inflammatory mediators for 24 h. Statistical analyses were performed using the Mann–Whitney *U* test or one-way ANOVA (CI 95%), with Tukey's *post hoc* testing.
Results
HRPE773 expression was significantly higher in amnion from normal term labour compared to caesarean section ($P < 0.05$), whilst no differences were observed in chorio-decidua, placenta or myometrium. HRPE773 expression was significantly up-regulated in ectocervical cells following treatment with IL1β or LPS ($P < 0.05$). Finally, HRPE773 immunoreactivity was localised primarily in epithelia of foetal membranes and cervix, myometrial endothelium and fibrotic lesions in placenta.
Conclusions
HRPE773 is normally expressed at low levels in female reproductive tissues, but up-regulation in normal term amnion and ectocervical cell lines treated with inflammatory mediators suggests a possible role during labour. Epithelial localisation of HRPE773 protein in reproductive tissues is consistent with an innate immune function.

DOI: 10.1530/endoabs.34.P336

P337

Maternal and cord blood serum IGF1 IGF binding protein-3, in asymmetrically small for gestational age neonates

Amrit Gupta[1], Nisha Singh[2] & Swasti Tiwari[1,2]
[1]Sanjay Gandhi Post Graduate Institute of medical sciences, Lucknow, Uttar Pradesh, India; [2]King georges Medical University, Lucknow, Uttar Pradesh, India.

This study was planned to investigate the relationship between birth weight and IGF1, IGF binding protein-3 (IGFBP3), in neonates with normal growth (appropriate for gestational age: AGA) and retarded growth (small for gestational age: SGA). A total of 200 mothers were recruited, 120 serum samples were maternal and collected between gestational age of 32-38 weeks, 80 were cord blood from SGA and AGA fetus collected soon after delivery. 98 maternal samples and 48 cord blood samples were analysed. IGF1, IGFBP3 were measured in maternal serum and venous cord blood at birth by chemi-lumnescent immunometric assay. Maternal serum IGF1, did not show correlations with birth weight, though IGBP3 was positively correlated. In contrast, there were significantly positive correlations between birth weight and venous cord blood IGF1, IGFBP3 ($P<0.001$). In conclusion, cord blood IGF1, IGFBP3 play an important role in the regulation of fetal and neonatal growth. It is likely that IGFBP3 in maternal blood and IGF1 and IGFBP3 play an important role in growth potential of fetus.

DOI: 10.1530/endoabs.34.P337

P338

The melanocortin system in the male reproductive axis

Monika Dowejko[1], Caroline Smith[1], Stephen Getting[1], Paul R Le Tissier[2] & Joanne F Murray[1]
[1]University of Westminster, London, UK; [2]Institute of Child Health, London, UK.

Melanocortin receptors (MC_S, MC_1–MC_5) are GPCRs, activated with different affinities by the melanocortin peptides (α-, β-, γ-MSH and ACTH). They are widely distributed throughout the body displaying a multitude of actions however their role in reproductive physiology is unclear. Previously, we have shown a reduction of pituitary hormone content and abnormalities in testes morphology in male MC_3 null mice. The aim of this study was to characterise MC_S expression in the male mouse reproductive axis and to investigate the effects of MC_S agonists/antagonists on *in vitro* testicular testosterone production (TP).

MC_S expression patterns were determined in mRNAs from hypothalami, pituitaries and testes of Balb/c and C57BL/6 male mice by two-step reverse transcriptase (RT) - quantitative (q) PCR and analysed using qBase^{plus}. To examine the functions of MC_S in gonads, testes of Balb/c and C57BL/6 mice were hemi-sected and incubated with the potent MC_3 and, to a lesser extent, MC_4 agonist ([D-TRP^8]-γ_2-MSH; 0.3–10 μg/ml) in the presence or absence of hCG (50 mIU/ml) and/or a MC_3/MC_4 antagonist and MC_1/MC_5 agonist (SHU9119; 10 μg/ml) for 5 h at 35 °C in air.

There were no significant differences in the pattern of MC_S expression between C57BL/6 and Balb/c. The most abundant MC in the hypothalamus and pituitary appears to be MC_3 whilst in the testis it was MC_2. TP in C57BL/6 mice is 50% less than in Balb/c: confirming published data that the former mice are hypoandrogenic. Activation of MC_3 and MC_4 using [D-TRP^8]-γ_2-MSH had no or inhibitory effects on basal TP in Balb/c and C57BL/6, respectively. The same ligand decreased TP in both strains in the presence of hCG. In contrast, SHU9119±hCG increased TP threefold compared to basal levels independently of ligand dose in Balb/c. This study suggests that activation of specific MC_S can alter TP however further work is required.

DOI: 10.1530/endoabs.34.P338

P339

Hyperandrogenism secondary to ovarian hyperthecosis masked by concurrent use of an aromatase inhibitor: a case report

Muhammad Khan[1], Rupa Ahluwalia[1], Susannah Shore[2], Alison Waghorn[2] & Jiten Vora[1]
[1]Link 7C, Department of Diabetes and Endocrinology, The Royal Liverpool & Broadgreen University Hospitals NHS Trusts, Liverpool, UK; [2]Department of Surgery, The Royal Liverpool & Broadgreen University Hospitals NHS Trusts, Liverpool, UK.

Backgound

We report a case of a 63-year-old postmenopausal female diagnosed with ovarian hyperthecosis masked by concurrent use of an aromatase inhibitor.

Following diagnosis of breast cancer in 2009, requiring mastectomy with adjunctive chemotherapy, she was commenced on anastrozole. Later she noted gradual onset of frontal balding and hirsutism. Biochemistry revealed elevated serum levels of testosterone 13.2 nmol/l (range: <1.9 nmol/l) and androstenedione 25.6 nmol/l (range: 1.0–8.5 nmol/l) with normal DHEA levels. Raised androgen levels were presumed to be a consequence of concurrent use of anastrazole, an aromatase inhibitor. Despite switching to tamoxifen her testosterone levels continued to rise (13.8 nmol). MRI pelvis showed a right adnexal mass without any hallmark diagnostic features. Selective venous catheterisation of ovarian veins confirmed a right to left androgen gradient. Histology following bilateral salpingo-oophorectomy showed focal mild stromal hyperplasia with hyperthecosis. Subsequently both testosterone and androstenedione returned within normal limits.

Discussion

Ovarian hyperthecosis underpins an abnormal production of androgens from luteinized theca cells within the ovary. Primarily in postmenopausal women, it is characterised by severe hyperandrogenism and insulin resistance. Patients present with slowly, progressive hirsutism and virilization with raised testosterone levels. Although elevated testosterone levels with hyperthecosis on imaging confirm diagnosis, additional investigations including ovarian venous sampling and GnRH agonist testing may be required. Treatment of hyperthecosis depends on age, degree of virilisation, and pregnancy goals. In postmenopausal women and premenopausal women not planning future pregnancies, treatment options are bilateral oophorectomy or long-term GnRH-agonist treatment. In premenopausal women, symptomatic treatment of hirsutism and ovulation induction for infertility may be indicated.

Summary

Ovarian hyperthecosis should be considered in post-menopausal women with hyperandrogenism. Furthermore, with concurrent use of aromatase inhibitors, it should be considered as a differential if testosterone levels fail to decline following discontinuation of potential incriminating drug.

DOI: 10.1530/endoabs.34.P339

P340

Two rare conditions in one patient: Fragile X and congenital adrenal hyperplasia

Ahmed Imran Siddiqi
University College London Hospital, London, UK.

Introduction

I present here a patient with congenital adrenal hyperplasia and Fragile X syndrome. The two conditions are rare and not known to be related to each other.

Clinical case

A 26-years-old lady was referred to Endocirne clinic with primary amenorrhoea. She complained of hirsutism and some facial acne since 14 years of age. To the best of her recollection her thelarche was around 12 years of age and pubic hair started to appear at 10 years of age. She did have a bit delayed milestones in childhood and was diagnosed with mild learning difficulties. She used to be a tall girl in her childhood in school but later on she was not much different from her friends. None of her parents had history of delayed puberty. She was the only child. She was under the care of gynaecology team since the age of 16 in view of amenorrhoea. She had a working diagnosis of PCOS but she had no menstrual bleeding with combined oestrogen-progesterone pill or medroxy-progesterone challenges. Her endometrial thickness on USS scans was 4–5 cm over the years. Her BMI was 33, no facial or body hirsutism, no central obesity, skin bruising or supra-clavicular pad of fat. Breast development was complete and pubic and axillary hair growth was normal. Her 17-OH progesterone was 36.6, SST showed a rise in 17-OH progesterone to 49.6, testosterone was 3.4, LH 2.8, FSH 4.2, oestrogen 157, chromosomal analysis returned 46 XX and mutation in FMR1 was found confirming Fragile X syndrome.

Treatment and conclusion

She was commenced on prednisolone to help reduce testosterone and progesterone levels which eventually should help her with her periods. She had a light bleed 3 months after the start of steroids. We arranged counselling for her in view of Fragile X syndrome. Congenital adrenal hyperplasia (CAH) is much less common than polycystic ovarian syndrome (PCOS) and there is overlap in the clinical symptoms of the two conditions. The diagnosis of PCOS should be revised if the initial treatment does not get the desired response.

DOI: 10.1530/endoabs.34.P340

Steroids

P341

Defining contralateral adrenal suppression in primary aldosteronism: implications for diagnosis and outcome

Gregory Kline, Valerian Dias, Benny So, Adrian Harvey & Janice Pasieka
University of Calgary, Calgary, Alberta, Canada.

Unilateral forms of primary aldosteronism (PA) should, by inference, have a contralaterally normal and therefore suppressed adrenal zona glomerulosa. However, there is no agreed definition of expected adrenal suppression. We hypothesized two biochemical definitions of adrenal suppression based upon measurements done during adrenal vein sampling. These were then applied to a PA–AVS-outcomes database to determine whether either definition proved useful for interpretation of AVS and/or prediction of hypertension resolution in operated cases. Definition 1 was the proportional suppression of the uninvolved/lowest $adrenal_{aldo/cortisol}$:$IVC_{aldo/cortisol}$ ratio both pre and post cortrosyn stimulation. Definition 2 was the absolute decrease in the uninvolved $adrenal_{aldo/cortisol}$ ratio post cortosyn. Applied to a cohort of 99 confirmed PA cases, Definition 1 proved highly predictive of lateralization when using post cortrosyn values with an ROC AUC of 0.958, $P<0.0001$ for $adrenal_{aldo/cortisol} : IVC_{aldo/cortisol} < 1.4$ as the optimal criterion (sensitivity 90% and specificity 94%). Definition 2 was less predictive of lateralization unless a stringent criterion of lowest-$adrenal_{aldo/cortisol}$ decrease to <1.0 was used, in which case specificity was 100% but sensitivity just 66%. For blood pressure outcomes in the surgical subgroup ($n=52$), hypertension resolution was most commonly seen among subjects with adrenal suppression by both definitions although there was significant overlap with subjects requiring ongoing medication. In conclusion, post cortrosyn suppression of the uninvolved adrenal:IVC ratio is a highly useful definition of adrenal suppression that accurately predicts unilateral PA. This may be particularly useful in a case where AVS fails to catheterize one of the adrenal veins but suppression is seen on the other side. Adrenal suppression may also predict blood pressure outcome however a much larger PA database is likely necessary to determine the relative contribution of this predictor.

DOI: 10.1530/endoabs.34.P341

P342

Monitoring outcomes in congenital adrenal hyperplasia: have we been neglecting testosterone?

Naomi Sakai[1] & Carlo Acerini[2]
[1]Luton and Dunstable University Hospital, Luton, UK; [2]Department of Paediatrics, Cambridge University, Cambridge, UK.

Background
Congenital adrenal hyperplasia (CAH) is a group of disorders caused by defects in one of the enzymes in the adrenal steroidigenic pathways. The most common form is 21-hydroxylase deficiency which leads to decreased production of cortisol and aldosterone and increased androgen production. Close monitoring of treatment is important so that adrenal suppression is achieved without excessive exposure to glucocorticoids. The current gold standard for monitoring in CAH is use of clinical parameters including height and bone age. Biochemical markers such as androstenedione, 17-hydroxyprogesterone (17-OHP) and testosterone can be used alongside clinical measurements in monitoring. We assessed the utility of different biochemical markers, particularly focussing on the feasibility of using testosterone as a biochemical marker in monitoring CAH.
Methods
Literature search and analysis of studies relating to monitoring CAH. Only studies relating to 'classical' CAH and 21-hydroxylase deficiency were included.
Conclusion
Monitoring of therapy in CAH is important in order to avoid the unwanted complications resulting from high androgen levels. Currently capillary blood spot 17-OHP levels are used alongside clinical parameters in monitoring. Testosterone concentrations can be measured accurately using liquid chromatography–tandem mass spectrometry (LC–MS/MS) and this method is suitable for routine clinical use. Further work is required to refine the technique for using capillary samples for testosterone quantification using LC–MS/MS in order for it to be used routinely in monitoring CAH.

DOI: 10.1530/endoabs.34.P342

P343

A large retrospective audit of adrenal incidentalomas

Sviatlana Zhyzhneuskaya, Nora Wuerdemann, Sath Nag, Vijayaraman Arutchelvam & Simon Ashwell
James Cook University Hospital, Middlesbrough, UK.

Objective
To determine whether people with an incidentally discovered adrenal mass are investigated according to regional and global standards (AACE guidelines); to determine the frequency of autonomous endocrine function.
Methods
We did a retrospective analysis of clinical, pathology, and radiology records from January 2007 to December 2011. Patients were included if they were seen in the outpatient clinic for evaluation of an adrenal mass >1 cm in size detected incidentally during radiological imaging for an unconnected problem between January 2007 and December 2011 inclusive.
Results
63 patients met the inclusion criteria (62% females; mean age 63 (range 34–88) years). Investigations were completed with the following frequencies: overnight 1 mg dexamethasone suppression test 65%; 24 h urine free cortisol 57% (one of these two tests was performed in 92%); 0900 h ACTH 19%. U&E's 97%; renin and aldosterone 87%; metanephrines or catecholamines 94%; DHEA, androstenedione or testosterone 76%; 17-hydroxyprogestrone 44%; oestradiol (men and post-menopausal women) 17%; CT assessment of adrenal attenuation and washout 84 and 5% respectively; appropriate follow-up imaging 78%.
21% patients had bilateral incidentalomas; in 90% of patients the final diagnosis was benign (in the remaining 10% the final diagnosis was not clearly recorded). In 24% patients autonomous adrenal hormone secretion was detected. Of autonomously secreting adenomas 73% were cortisol-secreting (60% subclinical and 13% clinical Cushing's), 20% were pheochromocytomas, and 7% were aldosterone-secreting. 11% patients underwent adrenal surgery.
Conclusion
In this large cohort 24% of adrenal incidentalomas showed autonomous hormone secretion. The frequency of autonomous cortisol secretion (18%) was higher than previous data but phaeochromocytoms (5%) and aldosterone-secreting adenomas (2%) were detected with similar frequencies to previous reports. No adrenal carcinomas were detected in this series.

DOI: 10.1530/endoabs.34.P343

P344

Biophenols-rich pomegranate extract intake inhibits salivary cortisol and 11β-HSD1 activity and improves overall quality of life scores in healthy volunteers

Angela Stockton & Emad Al-Dujaili
Queen Margaret Universty, Edinburgh, UK.

Background and aim
Biophenols can act as powerful antioxidants. Pomegranate (*Punica granatum*) provides a rich and varied source of biophenols with the most abundant being ellagitannins, tannins, anthocyanins, ellagic, and gallic acids. Recently, we have shown that pomegranate juice consumption may alleviate cardiovascular risk factors by reducing systolic and diastolic blood pressure and exercise-induced oxidative stress. The aim of study was to investigate the effect of pomegranate extract (PomeGreat Pomanox) supplementation on salivary stress hormones and quality of life in human volunteers.
Methods
A randomized placebo controlled double blinded parallel trial was conducted. Participants ($n=29$; 22 females and seven males) consented to take part in the study. Age ranged from 19 to 62 years and BMI from 18.6 to 32.5 kg/m^2. Each volunteer consumed either one pomegranate extract or placebo capsule of identical appearance with water after meal daily for 4 weeks. Pomegranate extract capsule (1.083 g) contains 650 mg Pomanox (350 mg biophenols). Dietary history, habits and the health related Quality of Life Questionnaire (Rand 36) were also recorded pre- and post-intervention. Salivary cortisol and cortisone levels (morning, noon, and evening) were also assessed by specific and sensitive ELISA methods.
Results
Pomegranate extract intake caused a significant drop of salivary cortisol levels (morning, $39.5\pm19.6\%$, $P<0.001$ and noon, $43.1\pm32.3\%$, $P=0.016$). Salivary cortisol:cortisone ratio was also significantly reduced (morning, 1.11 ± 0.51 to 0.55 ± 0.26, $P<0.001$; noon, 1.57 ± 0.85 to 0.75 ± 0.72, $P<0.001$; and evening,

1.22±0.9 to 0.74±0.59, $P=0.011$). Physical ($P=0.018$) and social functioning ($P=0.021$), pain ($P=0.003$), general health ($P=0.008$), and overall Quality of Life score ($P=0.007$) were significantly improved in those taking the pomegranate extract capsules. There was a slight increase in salivary cortisol and cortisol:cortisone ratio in those taking the placebo.

Conclusion

These results suggest that pomegranate extract intake rich in biophenols reduces salivary cortisol levels and 11β-HSD1 activity, and improves health related quality of life scores. Pomanox might prove to be beneficial for people suffering from chronic stress.

DOI: 10.1530/endoabs.34.P344

P345

Role of estrogen hormone in lipopolysaccharide: induced Alzheimer's disease in female rats; possible underlying mechanisms and modulation by progesterone hormone

O M Abdelwahed[1], M Gamal[1], Z Abdelwahab[1], M Zickri[1], W El Eraky[2] & D O Saleh[2]
[1]Cairo University, Cairo, Egypt; [2]National Research Center, Cairo, Egypt.

The incidence of Alzheimer's disease (AD) is higher in women than men. Our study aimed to investigate the behavioral, biochemical, and histological changes in lipopolysaccharide (LPS)-induced AD, to study effect of estrogen in AD clarifying the possible involved underlying mechanisms and its modulation by progestsserone.

Groups

Female albino rats were divided into control, AD, AD+ovariectomy (OVX), AD+OVX+estrogen replacement and AD+OVX+estrogen+progesterone replacement groups. Levels of activity using the activity cage, motor coordination using the rotarod, cognitive abilities using T-maze, serum TNFα, estrogen receptor α, Bcl-2 and Seladin-1 gene expression, MDA levels in brain tissue, histological and morphometric studies were estimated.

Results

Increased time in T-maze, decreased activity in activity cage, duration of rotations in rotarod, increased TNFα, decreased Seladin-1and Bcl-2 expression, increased MDA level, decreased ERα, increased area percent of dark nuclei and area of amyloid plaques in AD group. Increased time in T-maze, decreased duration of rotations in rotarod, non-significantly changed activity in activity cage, increased TNFα, decreased Seladin-1 expression, ERα expression and area percent of ER immunoexpression, Bcl-2 expression, increased area percent of dark nuclei, MDA level and area of amyloid plaques in AD+OVX group was shown. Estrogen decreased time in T-maze, increased activity in activity cage, duration of rotations in rotarod, decreased TNFα, increased Seladin-1, Bcl-2, and ERα expression and area percent of immunoexpression, decreased MDA level, area percent of dark nuclei and area of plaques either alone or in combination with progesterone.

Conclusion

LPS-induced AD produced deterioration of cognitive and motor functions which was further aggravated by OVX. Estrogen improved the cognitive and motor dysfunction partly through anti-inflammatory, anti-oxidant, anti-apoptotic and anti Aβ effects mediated partly through ER-α which was potentiated by co-administration of progesterone.

DOI: 10.1530/endoabs.34.P345

P346

A rapid and sensitive LC–MS/MS method for the simultaneous analysis of testosterone, androstenedione, 17-OHP, and DHEAS

Brian Keevil[1,2,3]
[1]University Hospital of South Manchester, Manchester, UK; [2]Manchester Centre for Endocrinology and Diabetes, Manchester, UK; [3]Manchester Academic Health Sciences Centre, Manchester, UK.

Introduction

Measurement of serum testosterone is necessary for the investigation of androgen disorders in males and females. Measurement of other androgens such as DHEAS and androstenedione (A*4*) may also be important in the investigation of PCOS and 17-hydroxyprogesterone (17-OHP) is useful to differentiate late onset CAH in patients with raised A*4*. Although it would be advantageous to measure all four steroids simultaneously this is not always easy because of their differing extraction requirements. We describe a simple method for the simultaneous measurement of testosterone, A*4*, 17-OHP, and DHEAS using LC–MS/MS after on line sample preparation.

Methods

Initial sample preparation involved the addition of serum (50 µl) to 150 µl $ZnSO_4$ and 100 µl acetonitrile (inc internal standards) to precipitate proteins. After centrifugation the supernatant was extracted further using on-line solid phase extraction by a Waters Acquity/on-line sample manager (OSM) coupled to a Waters Xevo TQ tandem mass spectrometer.

Results

Separation of all four steroids was achieved within a run time of 5.5 min/sample. The LLOQ for each assay was 0.1 nmol/l for testosterone, 0.25 nmol/l for A*4*, 0.1 nmol/l for 17-OHP, and 0.1 µmol/l for DHEAS.

The CV for each assay was <8% at concentrations of 0.4 nmol/l (testosterone), 1.7 nmol/l (A*4*), 0.6 nmol/l (17-OHP), and 0.2 µmol/l (DHEAS).

The comparison with validated assays ($n=102$) using either liquid/liquid extraction (LLE) or protein precipitation (PPT) gave the following equations: OSM (testosterone)=1.02×LLE (testosterone), $r^2=1.0$; OSM (A*4*)=1.01×LLE (A*4*), $r^2=0.99$; and OSM (DHEAS)=1.0×PPT (DHEAS)+0.54, $r^2=1.0$

Discussion

We have developed a rapid assay for the LC–MS/MS measurement of testosterone, A*4*, 17-OHP, and DHEAS. The assay is suitable for routine clinical use and the small sample volume is good for paediatric work. The assay demonstrated excellent performance compared to existing validated LC–MS/MS methods in our laboratory.

DOI: 10.1530/endoabs.34.P346

P347

A case of recurrent, re-admissions with severe hyperemesis gravidarum

Pushpa Jinadev[2] & Koshy Jacob[1]
[1]Pilgrim Hospital, United Lincolnshire NHS Trust, Boston, UK;
[2]Manchester Royal Infirmary, Manchester, UK.

Mrs XY, 29 years. (gravida: 3 and para 2) was admitted under the obstetricians with severe hyperemesis gravidarum (HG). An ultrasonogram revealed single live foetus of 8 weeks gestation. Her two previous pregnancies were normal with no history of HG. Mrs XY was on levothyroxine 150 µg OD for autoimmune primary hypothyroidism with TPO antibodies of 259 kU/l (0–5.6). She was also taking sertraline 200 mg OD for depression. The hyperemesis settled with i.v. fluids as well as antiemetic and she was discharged home.

Subsequently, Mrs XY had a further four admissions of varying duration to hospital for severe HG. At the last admission she was upset and expressed a desire to terminate her pregnancy. An endocrine consult was requested by the obstetricians for deranged thyroid function test. At the Endocrine consult, Mrs XY was noted to be hyperpigmented especially in elbows and buccal mucosa with a blood pressure of 100/59 mm Hg. Blood investigations revealed Na 136 mmol/l (133–146), K 3.8 mmol/l (3.5–5.3), urea 0.5 mmol/l (2.5–5.8), glucose 7.9 mmol/l (3–6), Hb 10.4 g/l (115–148), MCV 87fL (84–99), and eosinophil's were normal. TSH of 0.24 mU/l (0.35–4.94) with a free T*4* of 15.8 pmol/l (9–19.1). A random cortisol level was <20 nmol/l with a Short Synacthen test both basal as well as 30 min undetectable at <20 nmol/l. ACTH levels were elevated at >1250 ng/l. Adrenal antibodies were positive. Miss XY was treated as Addisonian crisis with i.v. hydrocortisone as well as i.v. fluids. She felt much better and later discharged with oral hydrocortisone and fludrocortisone. There were no further admissions with HG during the pregnancy and she delivered a healthy baby at 38 weeks gestation.

Discussion

A new diagnosis of primary adrenal insufficiency during pregnancy is rare but could become rapidly fatal if untreated. A high index of suspicion is necessary especially in patients with other autoimmune disorders like primary hypothyroidism. The hyperpigmentation of pregnancy 'chloasma' can cause diagnostic confusion but it usually occurs in sun exposed areas while the hyperpigmentation of Addison's occur in areas of mechanical friction as well as the mucosae. Recurrent severe vomiting, fatigue as well as a low blood pressure in the presence of hyperpigmentation should prompt investigations for an adrenal insufficiency in pregnancy.

DOI: 10.1530/endoabs.34.P347

P348

The effect of green tea extracts on steroidogenesis, proliferation and apoptosis in ovarian granulosa cells

Attila Kadasi[1,4], Nora Maruniakova[1], Adriana Kolesarova[1], Aneta Stochmalova[2,4], Richard Alexa[2,4], Roland Grossmann[3] & Alexander V Sirotkin[2,4]
[1]Department of Animal Physiology, Slovak University of Agriculture, Nitra, Slovakia; [2]Department of Zoology and Anthropology, Constantine the Philosopher University, Nitra, Slovakia; [3]Department of Functional Genomics and Bioregulation, Institute of Animal Science, Neustadt, Germany; [4]Institute for Genetics and Reproduction of Farm Animals, Animal Production Research Centre, Luzianky near Nitra, Slovakia.

The aim of our study was to examine the influence of green tea molecules on basic ovarian functions. For this purpose we have examine the effect of green tea extract, fraction of antioxidants (GTAO) and green tea fraction of polyphenols (GTPP) (at the doses 0, 1, 10, and 100 μg/ml) on proliferation, apoptosis, and steroidogenesis by cultured porcine ovarian granulosa cells. Markers of proliferation (PCNA) and apoptosis (bax) were detected by immunocytochemistry. Secretion of steroid hormones (progesterone and testosterone) was measured by RIA.
It was observed, that both GTAO and GTPP addition diminished the percentage of proliferative (PCNA-positive) granulosa cells and increased the occurence of apoptosis (proportion of bax-positive cells).
Addition of GTAO increased progesterone release and decreased testosterone. GTPP treatment stimulated progestin but reduced androgen.
These observations suggest that green tea antioxidants and polyphenols can affect basic ovarian cell functions. They can induce the suppression of proliferation, stimulation of apoptosis, promotion of progestagen and changes in testosteron release. The suppressive influence of green tea consumption on reproductive functions are therefore not to be excluded.

DOI: 10.1530/endoabs.34.P348

P349

Hyperandrogenaemia predicts metabolic phenotype in polycystic ovary syndrome: the utility of serum androstenedione

Michael O'Reilly[1], Angela Taylor[1], Nicola Crabtree[2], Beverly Hughes[1], Farfia Capper[3], Rachel Crowley[1], Paul Stewart[1], Jeremy Tomlinson[1] & Wiebke Arlt[1]
[1]CEDAM, University of Birmingham, Edgbaston, Birmingham, UK; [2]Department of Nuclear Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham, UK; [3]Wellcome Trust/NIHR Clinical Research Facility, Queen Elizabeth Hospital, Edgbaston, Birmingham, UK.

Polycystic ovary syndrome (PCOS) is a clinical triad of anovulation, insulin resistance, and androgen excess. Hyperandrogenism may correlate with metabolic risk but PCOS consensus criteria currently define androgen excess on the basis of serum testosterone only. Here we studied the utility of the androgen precursor serum androstenedione in conjunction with serum testosterone as a predictor of metabolic dysfunction in PCOS.
86 PCOS patients fulfilling Rotterdam diagnostic consensus criteria and 43 age- and BMI-matched controls underwent an oral glucose tolerance test (OGTT), with calculation of HOMA-IR and an insulin sensitivity index (ISI). Serum androgens were measured by liquid chromatography–tandem mass spectrometry (LC/MS). We analysed 24-h urine androgen excretion by gas chromatography/-mass spectrometry (GC/MS).
PCOS patients had higher levels of serum androgens and urinary androgen metabolites than controls (all $P<0.0001$). Within the PCOS cohort, both serum androstenedione and testosterone were positively correlated with the free androgen index and total androgen metabolite excretion (all $P<0.0001$). All PCOS subjects with testosterone above the normal reference range (high testosterone (HT)) also had high androstenedione (HA/HT group, $n=56$). The remaining 30 patients had normal testosterone levels, either in the presence of high androstenedione (HA/NT; $n=20$) or normal androstenedione (NA/NT; $n=10$). The groups did not differ in age or BMI. HA/HT and HA/NT had higher total androgen excretion than NA/NT ($P<0.01$ and $P<0.05$, respectively). The incidence of dysglycemia on OGTT increased with severity of androgen phenotype (NA/NT 0%; HA/NT 14%; HA/HT 25%, $P=0.03$). Multiple linear regression showed a strong negative association between serum androstenedione and insulin sensitivity index (coeff. −2.69, 95% and CI −1.57, −3.81), which was not observed with serum testosterone.
Simultaneous measurement of serum testosterone and androstenedione represents a useful tool for predicting metabolic risk in PCOS women. High androstenedione levels are a sensitive indicator of PCOS-related androgen excess.

DOI: 10.1530/endoabs.34.P349

P350

Upregulation of subcutaneous adipose AKR1C3 expression in obese females: evidence for depot- and sex-specific effects

Michael O'Reilly, Laura Gathercole, Wiebke Arlt & Jeremy Tomlinson
CEDAM, University of Birmingham, Edgbaston, Birmingham, UK.

Polycystic ovary syndrome (PCOS) is a clinical triad of anovulation, insulin resistance, and androgen excess in women. Adipose androgen generation of testosterone from androstenedione by aldo-ketoreductase type 1C3 (AKR1C3) may contribute to hyperandrogenism, particularly in the setting of obesity. We aimed to determine the effects of BMI and age on AKR1C3 expression in subcutaneous (SC) and omental (OM) fat depots in both women and men.
Paired SC and OM samples were obtained from men and women undergoing abdominal surgery at the Queen Elizabeth Hospital Birmingham. Baseline demographic data including age and BMI was collected. RNA was extracted; after RT, AKR1C3 mRNA expression was determined by realtime quantitative PCR. Pearson's correlation testing was used to determine relationships between variables.
Paired SC and OM samples from 38 women and 23 men (total 122 samples) were analysed (mean age 56.4 ± 1.7 and 60 ± 2.6 years respectively). Mean BMI was 28.8 ± 0.8 kg/m^2 in women and 27.8 ± 0.5 kg/m^2 in men. AKR1C3 expression (ΔCT) was similar in men and women (8.1 ± 0.5 and 8.3 ± 0.6 respectively). Expression was significantly higher in SC compared to OM fat (7.1 ± 0.6 vs 9.2 ± 0.4, $P=0.004$), and in obese compared to non-obese women (6.4 ± 1.1 vs 8.9 ± 0.4, $P=0.01$). BMI had a strong positive correlation with SC adipose AKR1C3 expression in women ($R=-0.51$ for ΔCT, $P=0.006$); this association was not observed in men or in omental fat. AKR1C3 expression declined with age in men but not women ($R=0.61$ for ΔCT, $P=0.009$).
We have found evidence of depot- and sex-specific regulation of AKR1C3 by BMI and age in human adipose tissue. Subcutaneous AKR1C3 expression is upregulated in obesity in females. Obesity may drive adipose androgen generation in PCOS through this pathway.

DOI: 10.1530/endoabs.34.P350

P351

High 11β-HSD1 activity is associated with progression to rheumatoid arthritis in patients first presenting with inflammatory arthritis

Dominika Nanus[1,2], Andrew D Filer[1,3], Lorraine Yeo[1], Dagmar Scheel-Toellner[1], Rowan Hardy[2], Gareth L Lavery[2], Paul M Stewart[5], Christopher D Buckley[1,4], Mark S Cooper[6] & Karim Raza[1,4]
[1]Rheumathology Research Group, University of Birmingham, Birmingham, UK; [2]Centre for Endocrinology, Diabetes and Metabolism, University of Birmingham, Birmingham, UK; [3]Rheumathology, University Hospital Birmingham NHS Foundation Trust, Birmmingham, UK; [4]Rheumathology, Sandwell and West Birmingham Hospitals NHS Trust, Birmingham, UK; [5]University of Leeds, Leeds, UK; [6]ANZAC Research Institute, Concord Repatriation General Hospital, University of Sydney, Sydney, New South Wales, Australia.

Variation in endogenous glucocorticoid (GC) activity during inflammation has been linked to susceptibility to developing rheumatoid arthritis (RA). It has been shown that in patients with RA inflamed synovial tissue can generate active GCs through the expression of the 11β-hydroxysteroid dehydrogenase type 1 enzyme (11β-HSD1), which converts cortisone to cortisol. We examined whether the total body activity of 11β-HSD1 or its expression within synovium was associated with the risk of developing persistent arthritis in patients first presenting with joint inflammation. Blood, urine, and synovial tissue biopsies were obtained from 76 patients at first presentation with arthritis. Total body 11β-HSD1 activity was determined by urinary gas chromatography/mass spectrometry and calculated as the tetrahydrocortisol + allotetrahydrocortisol/tetrahydrocortisone ((THF + alloTHF)/THE) and the cortols:cortolones ratios. Urinary 11β-HSD2 activity was measured as the UFF:UFE ratio. Synovial tissue expression of 11β-HSD1 and 11β-HSD2 was assessed by qPCR. Arthritis severity was assessed by ESR, CRP, and DAS28. Total body 11β-HSD1 activity was significantly lower in patients with arthritis that subsequently resolved than in patients with arthritis that went on to develop into persistent arthritis (Persistent RA, 1.34 (0.013) and Resolvers, 0.96 (0.07), $P=0.012$). Similar changes were seen in the cortols:cortolones ratio ($P=0.0002$). There was no difference in renal 11β-HSD2 activity between patients who resolved and those that developed persistent arthritis. Despite the difference in total body 11β-HSD1 activity there was no significant difference between groups in synovial tissue 11β-HSD1 expression. There was no difference in ESR or DAS28 between patients that resolved or went on to persistence although resolvers had a lower level of CRP. These studies demonstrate that a high total body 11β-HSD1 activity during early arthritis is associated with a

reduced probability of resolution. The excess 11β-HSD1 may have an articular and/or extra-articular origin. This work raises the possibility that targeting 11β-HSD1 activity in early arthritis could impact on the development of RA.

DOI: 10.1530/endoabs.34.P351

P352

Urinary steroid profiling by Gas-Chromatography Mass-Spectrometry (GC–MS) is not helpful in the diagnosis of Cushing's Syndrome

Emma Johns[1], David Shapiro[3], Frances McManus[2] & Marie Freel[1,2]
[1]Department of Endocrinology, Western Infirmary, Glasgow, UK; [2]Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK; [3]Department of Clinical Biochemistry, Glasgow Royal Infirmary, Glasgow, UK.

Introduction
The diagnosis of Cushing's syndrome (CS) can be challenging and there is no single gold standard diagnostic test. Endocrinologists rely on a combination of plasma cortisol (before and after dexamethasone), midnight salivary cortisol and urinary free cortisol (UFC) to make the diagnosis. Assessment of urinary corticosteroids and their metabolites, measured by gas chromatography–mass spectrometry (GC–MS), provide a comprehensive picture of corticosteroid production, metabolism and excretion and so this may be a helpful additional diagnostic tool in CS.
Methods
We retrospectively examined the urinary steroid profiles assessed before and after a low-dose dexamethasone suppression test of 23 patients referred to our unit for further evaluation of possible CS. Patients were labelled as CS ($n=10$) or non-CS ($n=13$) by a consultant endocrinologist on the basis of clinical suspicion, UFC and post-dexamethasone plasma cortisol.
Results
Patients with CS demonstrated elevation only of glucocorticoid metabolites (tetrahydrocortisol (THF)/tetrahydrocortisone (THE)/allo THF/cortol and cortolone) with no significant suppression by dexamethasone. There was no difference in baseline steroid pattern between ACTH ($n=6$) and non ACTH ($n=4$) dependent CS. All urinary metabolites were within normal range in non-CS patients; glucocorticoids and mineralocorticoids (not androgens) were significantly suppressed after dexamethasone ($P<0.03$ in all cases). The only significant difference in urinary steroids between these groups was elevated post-dexamethasone glucocorticoid metabolites in CS subjects ($P<0.03$). Plasma cortisol correlated strongly with urinary total cortisol after dexamethasone suppression in both patient cohorts ($P<0.01$ in both groups).
Conclusions
Urinary steroid profiling using GC–MS provides no diagnostic advantage over conventional dexamethasone suppression testing +/− UFC in CS and should not be routinely performed in this setting. Whether it may help in subtype differentiation of CS requires further investigation. Currently, its use should remain limited to biochemical phenotyping of adrenal adenomas.

DOI: 10.1530/endoabs.34.P352

P353

A novel UPLC–MS/MS method to extract and quantify sulphated and non-sulphated oestrogens automatically optimised using MUSCLE software

Lorna Gilligan, James Bradbury, Angela Taylor, Shan He, Donna O'Neil, Mark Viant & Paul Foster
University of Birmingham, Birmingham, UK.

Oestradiol (E_2) and oestrone (E_1) are implicated in many diseases and drive cell proliferation in breast, ovarian, and endometrial cancer. Inactive sulphated oestrogens (E_2S and E_1S) represent a circulating reservoir forming active de-sulphated oestrogens. Therefore, new methods that accurately measure both sulphated and non-sulphated oestrogen concentrations have potential value for understanding oestrogen-related cancer. We present here a novel mass spectrometry method that extracts and quantifies both oestrogens and their sulphates together.
The method used was a development of a method Owen *et al.* (2013). Samples with representative internal standards were extracted with methanol using Isolute solid phase extraction columns and analysed by Waters Xevo LC–MS/MS in negative ion mode with 0.3 mM ammonium fluoride (aqueous phase). Separation of these four steroids was further optimised using MUSCLE software. The MUSCLE software (Multi-objective Unbiased optimisation of Spectrometry via Closed Loop Experimentation) has recently been developed by Bradbury *et al.* and enables robust, objective and automated optimisation of targeted LC–MS/MS analyses, as described at www.muscleproject.org
The MUSCLE software optimised method analysed E_1, E_2, E_1S and E_2S over the linear range 0.5–500 ng/ml with a run time of 6.5 min. For E_1 and E_2 the CV at 18 nmol/l was 5 and 15% respectively and at 554 nmol/l 5 and 4% respectively. For E_1S and E_2S this assay demonstrated a CV at 14 nmol/l of 9 and 14% respectively and at 427 nmol/l 9 and 8% respectively. The average recovery for all oestrogens was ∼100%.
This novel method involves rapid extraction and analysis to accurately quantify E_1, E_2 and their sulphates and represents a significant improvement on previous methodologies. This method can be applied to both cell culture medium and serum based research, and will be a significant benefit to future oestrogen-related research.

DOI: 10.1530/endoabs.34.P353

P354

Dexamethasone-related adrenal insufficiency in patients with solid brain tumours

Helen Currie & Muzzammil Ali
University of Birmingham, Birmingham, UK.

Dexamethasone is used to reduce cerebral oedema thus relieve symptoms of raised intracranial pressure. Long-term use causes suppression of the hypothalamic–pituitary–adrenal axis, which can lead to adrenal insufficiency. Undiagnosed, significant morbidity and mortality can occur from Addisonian crisis. The aim of this audit was to discover the relationship between adrenal insufficiency and long-term steroid use, in order to make recommendations for the use and withdrawal of dexamethasone in patients with solid brain tumours.
Patients who had a Short Synacthen test (SST) who also attended brain tumour clinic at the University Hospital Birmingham formed the basis of the study sample. This sample was divided into two groups depending on whether they passed the SST or failed it (i.e. have adrenal insufficiency). A retrospective evaluation of patient records was carried out and an area under the curve was calculated for each patient.
Results showed a significant difference between those who passed and failed their SST, but no differences within the groups. A significant negative correlation existed between baseline cortisol and both steroid duration and exposure, as well as between 30 min cortisol and duration, dose and exposure. Within the pass group subjects with no exposure were then excluded, statistically the group was still the same. A significant difference still existed between pass and fail groups. Results suggested a threshold point for failing the SST. Values at which failing the SST are most likely and significant are 2 mg mean dose, 150 day duration and 450 mg days exposure.
Therefore, steroid use above these threshold levels are most likely to cause adrenal insufficiency. With these findings, it can be recommended that such patients don't need an SST; they are high risk of adrenal insufficiency, so should have hydrocortisone therapy to facilitate withdrawal of dexamethasone.

DOI: 10.1530/endoabs.34.P354

P355

Patient education and steroid replacement regimens in adrenal insufficiency

Zana Ameen, Sadaf Ulnasah, Jo Rees, Lisa Shepherd, Andrew Bates & Asad Rahim
Heart of England NHS Foundation Trust, Birmingham, UK.

Introduction
Adrenal insufficiency (primary/secondary) is a rare and potentially life-threatening condition which can be easily overlooked. Patient awareness and management of problems is critical and central to effective treatment. The NICE Clinical Knowledge Summaries for patients identifies and recommends critical areas that require attention in adrenal insufficiency. In particular, patients should: i) Realize the need for lifelong glucocorticoid replacement treatment. ii) Know how to adjust their replacement steroid medication. iii) Know how to recognize the symptoms of an adrenal crisis and how to give intramuscular hydrocortisone in an emergency (family member should also know). iv) Understand the importance of carrying emergency information.
The aim of this audit was to determine if further strategies need to be implemented for our practice to comply with guidelines.

Method
In a retrospective analysis of 71 patients with adrenal insufficiency we reviewed clinical documentation and objective data (clinical observations, biochemical results and educational information including emergency hydrocortisone injection kits).
Results
We found that:
i) 100% of patients received complete education as recommended including issuing of emergency hydrocortisone injection kits.
ii) 99% had medication documented with 67/71 (94%) on hydrocortisone and 44/67 (66%) taking 10-5-5. Eleven of 67 (16%) on higher doses, 7/67 (10%) on lower doses, 5/67 (7%) no dose recorded. Two patients were taking Plenadrin (20 and 15 mg) and two on Prednisolone.
iii) 54% had a blood pressure recording, 17% above 140/80. 60% had U&E's recorded.
Conclusion
For good practice, clinicians must ensure all patients with adrenal insufficiency receive education about the importance of steroid replacement. This can be provided in group sessions or on a 1:1 basis by Endocrine Nurses. The majority of patients were on the recommended replacement regimen. However, we will need to review those on other regimens to ensure these are appropriate and tailored to patient's needs.

DOI: 10.1530/endoabs.34.P355

P356

Evaluating progesterone as a novel neuroprotective drug after ocular injury

Eleanor Mccance & Richard Blanch
University of Birmingham, West Midlands, UK.

Background
Blunt ocular trauma causes commotio retinae, a retinal opacification that accounts for over a third of all retinal injuries. Poor outcomes occur when the macula is affected, which accounts for 1/3 of cases and 3/4 cases in those at high injury risk, causing permanent visual loss due to selective photoreceptor death. This is seen in humans, and reproduced in our animal model, as loss of the outer nuclear layer (ONL) on histological and optical coherence tomography (OCT) images.
Photoreceptor apoptosis may be prevented and visual outcomes improved by manipulation of cell death pathways using neuroprotective agents. Progesterone has well-established neuroprotective effects in various models of CNS injury and there is growing evidence that its pleiotropic actions that interfere with cell death pathways may be extended to ocular trauma.
Methods
The present study aimed to assess the neuroprotective efficacy of progesterone for photoreceptor damage in our experimental model of commotio retinae with respect to histological and functional outcomes. Progesterone was administered intraperitoneally (8 mg/kg bolus dose) immediately after trauma, with subsequent continuous infusion (5 µl/h of 10 µg/µl solution) over a 2-week experimental period via osmotic minipump, aiming to reach supraphysiological plasma progesterone levels, above those seen in pregnant rats, where a neuroprotective effect has been observed.
Results
Progesterone induced significantly greater photoreceptor death ($P=0.002$), with a more pronounced negative effect apparent at increasing distances from the impact site. ERG findings show an initial enhancement of photoreceptor function after 7 days, but overall deterioration after 14 days ($P=0.005$).
Discussion
This is the first study to report a differential effect of progesterone after short and longer term end points. Progesterone caused a delayed heightened wave of photoreceptor apoptosis after ocular trauma.
Conclusion
Whether this was a concentration, or dosage duration-dependent effect requires further investigation.

DOI: 10.1530/endoabs.34.P356

P357

Steroid sulfatase contributes to systemic androgen activation in pre-pubertal boys: lessons from steroid sulfatase deficiency

Jan Idkowiak[1,4], Angela E Taylor[1], Donna M O'Neil[1], Sandra Subtil[1], Raymon Vijzelaar[2], Renuka P Dias[3,4], Rakesh Amin[5], Timothy G Barrett[1,4], Jeremy W Kirk[4], Cedric H L Shackleton[1], Celia Moss[6] & Wiebke Arlt[1]
[1]Centre for Endocrinology, Diabetes and Metabolism, University of Birmingham, Birmingham, UK; [2]MRC Holland, Amsterdam, The Netherlands; [3]Centre for Rare Diseases and Personalized Medicine, University of Birmingham, Birmingham, UK; [4]Department of Paediatric Endocrinology, Children's Hospital Birmingham NHS Foundation Trust, Birmingham, UK; [5]Department of Paediatric Endocrinology, Great Ormond Hospital for Children NHS Trust, London, UK; [6]Department of Paediatric Dermatology, Children's Hospital NHS Foundation Trust, Birmingham, UK.

Steroid sulfatase (STS) cleaves the sulfate moiety off steroid sulfates, including DHEAS, the inactive sulfate ester of the adrenal androgen precursor DHEA. Deficient DHEA sulfation, the opposite enzymatic reaction to that catalysed by STS, results in androgen excess by increased conversion of DHEA to active androgens. STS deficiency (STSD) due to deletions or inactivating mutations in the X-linked *STS* gene manifests with ichthyosis, but androgen homeostasis in STSD has not been studied in detail yet.
Here, we have investigated 30 male patients with genetically confirmed STSD (age 6–30 years) and 45 age- and sex-matched healthy controls including detailed clinical and genetic assessment and serum and 24 h urine steroid analysis by mass spectrometry (GC/MS and LC/MSMS).
Multiplex-ligand probe-amplification (MLPA) revealed that 27/30 (90%) of the STSD patients had complete, isolated deletions of the *STS* gene; one patient had a partial deletion of exon 7 and two patients had a known, disease-causing missense mutation confirmed by Sanger sequencing. There were no apparent abnormalities in physical development and pubertal progression in STSD patients. Urinary excretion of active androgen metabolites did not differ in STSD and controls. However, serum testosterone levels in the post-pubertal subgroup were lower than in controls ($P=0.03$), albeit in the normal range. DHEAS and sulfated androgen precursor metabolites were higher in STSD but DHEA was lower. The ratios of serum DHEA:DHEAS, reflecting STS activity, in pre-/peri-pubertal controls were significantly higher than in post-pubertal controls whereas all STSD patients had low DHEA:DHEAS ratios. In addition, STSD patients had a higher ratio of the 5α-reduced glucocorticoid precursor 5α-tetrahydrocortisol over tetrahydrocortisol, indicating enhanced global 5α-reductase activity, possibly compensating for slightly lower testosterone levels via enhanced androgen activation. The finding of a higher DHEA:DHEAS ratio in healthy subjects before puberty suggests a physiological role of STS in androgen regulation prior to but not after puberty.

DOI: 10.1530/endoabs.34.P357

P358

Glucocorticoid receptor interactome

Emyr Bakker[1], Kun Tian[2], James Andrews[2], Constantinos Demonacos[2], Jean-Marc Schwartz[2] & Marija Krstic-Demonacos[1]
[1]University of Salford, Salford, UK; [2]University of Manchester, Manchester, UK.

Glucocorticoid hormones are used in the treatment of variety of diseases, due to their diverse range of effects. Acute lymphoblastic leukaemia (ALL), the most common form of childhood cancer, is one such disease treated by glucocorticoids (GCs). Although there has been much success in the treatment of ALL with GCs, drug resistance remains a problem, despite the detailed knowledge of the signalling networks underlying the GC actions through the glucocorticoid receptor (GR). Systems biology offers the opportunity to gain a holistic view of protein interaction networks, whilst also allowing for the generation of predictions through *in silico* knockouts which can be validated through conventional laboratory approaches. Systems biology has shown great success in identifying the functions of seemingly-redundant proteins. Here we present the beginnings of a model of the GR interactome, constructed through MATLAB using the add-on CellNetAnalyzer, and visualised through Cytoscape. The model currently consists of proteins that interact with the GR and interactions between these proteins. Protein interactions were extracted automatically from the STRING database using a Java program, and then manually curated by literature mining to remove false positives. The curated interactions were imported into CellNetAnalyzer. Future work will be directed towards expanding and connecting this model to drugs or processes like apoptosis, to detecting potential therapeutic targets, whilst also validating it through analysis of microarray data from ALL patients, paving the way for personalized treatment.

DOI: 10.1530/endoabs.34.P358

P359

Does recurrent hypoglycaemia, a known activator of the HPA axis, alter the diurnal pattern of cortisol release?

Priya George[1,2], Alasdair Mackie[2], John Connell[2] & Rory McCrimmon[1,2]
[1]Medical Research Institute, Ninewells Hospital and Medical School, Dundee, UK; [2]Ninewells Hospital and Medical School, Dundee, UK.

In population studies, premature cardiovascular disease is associated with cortisol dysregulation. Recently, recurrent hypoglycaemia in individuals with type 1 diabetes (T1D) was reported to be associated with increase carotid intima–media thickness. Hypoglycaemia is a potent activator of the hypothalamo-pituitary–adrenal axis (HPA axis) with eventual release of cortisol. We hypothesized that individuals with T1D who experience recurrent hypoglycaemia might demonstrate abnormal cortisol diurnal rhythms.

Data are presented on 100 subjects ($N=74$ T1D, $N=26$ non-diabetic, with each group matched for BMI, age, and sex) who participated in an on-going study examining the association between increased blood glucose variability and 24 h cortisol profiles as assessed by salivary cortisol. Each subject underwent a period of 6–7 days continuous glucose monitoring to assess glucose variability and in addition provided seven spit samples spaced through the day, to measure the free unbound cortisol.

T1D with high exposure to hypoglycaemia (low blood glucose Index (LBGI) >5) showed a non-significant trend to a greater cortisol AUC compared to control subjects (median AUC of control vs high LBGI was 9054 vs 10 350 with $P=0.0871$). There is also a non-significant reduction (1.2 vs 1.5 with $P=0.38$) in the slope of the cortisol decline, in those with high exposure to hypoglycaemia compared to controls. The AUC (g) measure was used to look at the morning cortisol rise. Those with high exposure to hypoglycaemia, also had a non-significant trend in lower AUC(g) as compared to the control group (47 vs 57 with $P=0.39$).

This study is still recruiting, however, these preliminary results suggest that increased glucose variability with a greater exposure to hypoglycaemia in T1D is associated with cortisol dysregulation which may in turn increase propensity to other conditions associated with hypercortisolaemia such as insulin resistance, cardiovascular morbidity, and psychological disturbances.

DOI: 10.1530/endoabs.34.P359

P360

Systemic availability of placental growth factor correlates with urinary tetrahydroaldosterone excretion in normal and preeclamptic pregnancy

Gemma Currie[1], Nicole Eisele[2], Heather Small[1], Genevieve Escher[2], Carine Gennari-Moser[2], David Carty[1], Markus Mohaupt[2] & Delles Christian[1]
[1]Institute of Cardiovascular and Medical Sciences, BHF Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, UK; [2]Department of Nephrology and Hypertension, Bern University Hospital, Bern, Switzerland.

Background

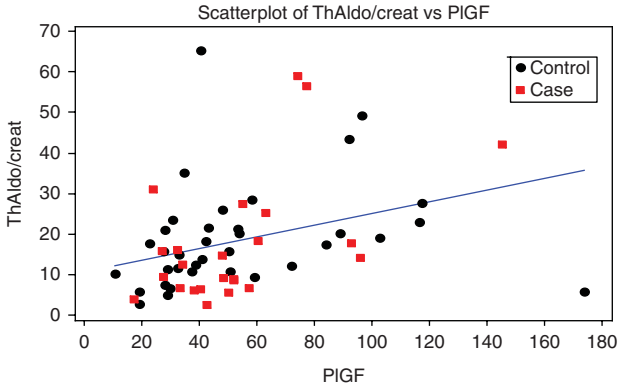
Aldosterone levels are elevated in normal pregnancy but fall despite volume contraction in preeclampsia. Vascular endothelial growth factor (VEGF) and soluble fms-like tyrosine kinase 1 (sFlt-1) have been implicated in this phenomenon *in vitro* and in animal work. Low placental growth factor (PlGF), which is closely linked to VEGF signalling, identifies women at risk of preeclampsia between 20 and 35 weeks gestation. We aimed to examine these relationships in human subjects in early pregnancy.

Methods

We examined levels of aldosterone and PlGF at gestational weeks 14–16 in women who had previously taken part in the Proteomics in Preeclampsia (PIP) Study; a longitudinal study of 2500 pregnancies investigating early pregnancy biomarkers to predict preeclampsia. Stored urine and plasma samples were obtained from 48 cases and 48 matched pregnant controls. Urinary tetrahydroaldosterone (THAldo) excretion was measured by gas chromatography–mass spectrometry (GC–MS) and PlGF by ELISA.

Results

Age, BMI, and booking systolic blood pressure were similar between groups. In line with findings of the PIP study, booking diastolic blood pressure was higher among women who went on to develop preeclampsia (72 mmHg, IQR 54–82, cf. 70 mmHg, IQR 56–78 by Mann–Whitney, $P=0.0452$.) Urinary THAldo:creatinine ratio was significantly lower among cases than controls (9.22 µg/mmol, IQR 1.41–16.6, cf. 15.96 µg/mmol, IQR 2.76–22.61 by *t*-test, $P=0.024$). Whilst the groups did not show any significant difference in early pregnancy PlGF level, we identified a linear relationship between PlGF and THAldo in both cases and controls (Spearman's rank correlation 0.415, $P=0.001$).



Conclusion

THAldo and PlGF are closely linked in early pregnancy. Given the critical role of THAldo in pregnancy, these data suggest that in women destined to develop preeclampsia low THAldo rather than PlGF is causal extending the findings of previous animal work to human subjects.

DOI: 10.1530/endoabs.34.P360

P361

Endogenous androgen-mediated modulation of neointima formation is independent of vascular androgen receptor

Junxi Wu[1,2], Patrick Hadoke[2], Iris Mair[2], Win Gel Lim[2], Eileen Millar[2], Laura Milne[1], Martin Denvir[2] & Lee Smith[1]
[1]The Queen's Medical Research Institute, MRC Centre for Reproductive Health, The University of Edinburgh, Edinburgh, UK; [2]The Queen's Medical Research Institute, BHF Centre for Cardiovascular Science, The University of Edinburgh, Edinburgh, UK.

Aims

Low circulating testosterone levels are associated with increased cardiovascular risk and there is evidence that androgens inhibit arterial lesion formation. This investigation addressed the hypothesis that androgens directly inhibit neointimal lesion formation by stimulation of vascular androgen receptor.

Methods and results

Mice were generated with selective deletion of androgen receptor from vascular (endothelial or smooth muscle) cells. Castration in WT mice produced a modest increase in neointimal lesion formation following denuding (wire) injury of the mouse femoral artery (sham vs castration: $2.4\times107\pm4.5\times106$ vs $3.9\times107\pm4.9\times106$ µm^3, $P=0.0386$, $n=9$–10), but not after non-denuding (ligation) injury. Pharmacological androgen replacement (testosterone, 10 mg/kg per day, via SUBCUTANEOUS minipump) in castrated mice did not, however, reduce lesion size (castration vs testosterone: $3.5\times107\pm3.1\times106$ vs $3.0\times107\pm3.2\times106$ µm^3, $P=0.2395$, $n=8$–9). Selective deletion of androgen receptor from vascular cells had no effect on circulating testosterone levels or seminal vesicle weight but produced a modest increase (~8–12 mmHg) in blood pressure. Deletion of androgen receptor from smooth muscle cells reduced (5.3 ± 0.33 vs 2.8 ± 0.55 mN/mm, $P<0.0001$, $n=7$–9) phenylephrine-mediated contraction in isolated femoral arteries. Selective deletion of androgen receptor from endothelial cells, smooth muscle cells or both did not alter the size of neointimal lesions generated by denuding or non-denuding arterial injury.

Conclusion

Neointimal lesion formation following denuding injury to the femoral artery is inhibited by endogenous androgens, independent of the vascular androgen receptor.

DOI: 10.1530/endoabs.34.P361

P362

An audit on the outcomes of bloodspot 17-OHP results on the management of patients with CAH

Erin Mozley, Simon Salter, Justin Davies & Annie Armston
University Hospital Southampton NHS Foundation Trust, Southampton, UK.

Introduction
Measurement of bloodspot 17-OHP is the current method of assessing hydrocortisone replacement in patients with congenital adrenal hyperplasia (CAH). Bloodspot 17-OHP has been measured in Southampton by LC–MS/MS since August 2011. Clinical observations of signs and symptoms of under- and over-treatment are equally as important.
The aim of this audit was to assess whether the 17-OHP profile results are being appropriately used for adjustments to hydrocortisone in patients with CAH by the Paediatric Endocrinologists in Southampton.
Method
17-OHP profile results were collected from August 2011 to June 2013, excluding patients not treated by the Southampton Endocrinologists. Clinic notes and letters were used to obtain information regarding clinical observations and changes to hydrocortisone dosage.
The data was compared to 17-OHP guidelines used by the Endocrinologists in Southampton, as well as ranges quoted by Cardiff University Hospital, to assess any potential differences in outcome. The expected outcomes based on these ranges were compared to the actual outcomes.
Results
A total of 54 profiles were collected, from 31 patients with CAH. The 17-OHP results and patient symptoms agreed with the outcome in 76% cases. In 22% the hydrocortisone dose was changed without clinical indication, but the change was based on the 17-OHP results. In one case, the 17-OHP profile and clinical symptoms suggested an increase in dosage was required, but no change was made. On comparison to the Cardiff guidelines, there was a difference in interpretation in 30% compared to the Southampton guidelines, but in all cases the outcome was appropriate based on either the 17-OHP or the clinical details.
Discussion
The Southampton 17-OHP ranges were adhered to in 98% of cases. These guidelines are appropriate as any medication adjustments have been clinically well-tolerated with no adverse effects. There was no difference in expected outcome when compared to the Cardiff guidelines.

DOI: 10.1530/endoabs.34.P362

P363

Androgen receptor over expression drives lipid accumulation in human hepatocytes

Nikolaos Nikolaou[1], Maryam Nasiri[1], Laura Gathercole[1], Silvia Parajes Castro[1], Nils Krone[1], George Valsamakis[2], George Mastorakos[2] & Jeremy Tomlinson[1]
[1]University of Birmingham, Birmingham, UK; [2]National and Kapodistrian University of Athens, Athens, Greece.

Non-alcoholic fatty liver disease NAFLD has been associated with androgen deficiency, yet in the majority of patients with non-alcoholic steatohepatitis NASH, androgens levels are normal. In contrast, women with polycystic ovarian syndrome PCOS, which is characterised by androgen excess, have evidence of increased liver fat. Our hypothesis is that androgen exposure to the liver may be crucial in the amount of lipid that can accumulate in hepatocytes. C3A human hepatoma cells were cultured and treated with testosterone testo (5 and 50 nM) or dihydrotestosterone DHT (1 and 10 nM) for 24 h. Lipid accumulation was measured by C^{14} acetate incorporation and gene expression by real-time PCR. Cells were transfected with an androgen receptor AR construct (pcDNA3.1 + AR) or vector as a control. FASN, ACC1, ACC2, and CPT1 mRNA expression was significantly increased after dose dependent treatment with testosterone and DHT (Table 1) and this was further augmented after AR over expression (FASN: ctrl 13.90 ± 1.99 vs AR 66.78 ± 6.22, ACC1: ctrl 1.06 ± 0.26 vs AR 3.52 ± 0.29, ACC2: ctrl 0.49 ± 0.11 vs AR 1.02 ± 0.09, and CPT1: ctrl 1.78 ± 0.25 vs AR 4.33 ± 0.22, $P < 0.05$). Both testosterone and DHT increased *de novo* lipogenesis and this was also increased following AR over expression (7001.8 ± 258.58 ctrl vs 8.747.76 ± 433.39 Testo, 8.970.03 ± 330.17 DHT, and AR 14193.2 ± 755.17 AR, $P < 0.05$). In conclusion, these data highlight that enhanced androgen action is able to drive lipid accumulation in human hepatocytes and this may be crucial in understanding the association between PCOS and NAFLD.

Gene of interest	Control (mean AU ± S.E.M)	5 nM Testosterone (mean AU ± S.E.M)	50 nM Testosterone (mean AU ± S.E.M)	P value (5 nM/50 nM)
FASN	13.90 ± 1.99	41.97 ± 5.80*	60.36 ± 3.34*	0.05/0.008
ACACA (ACC1)	1.05 ± 0.26	2.03 ± 0.56	3.06 ± 0.41*	0.246/0.016
ACACB (ACC2)	0.50 ± 0.11	0.78 ± 0.14	0.96 ± 0.15	0.955/0.15
CPT1	1.78 ± 0.25	1.92 ± 0.14	4.19 ± 0.89*	0.89/0.039
Gene of interest	**Control (mean AU ± S.E.M)**	**1 nM of DHT (mean AU ± S.E.M)**	**10 nM DHT (mean AU ± S.E.M)**	**P value (1 nM/10 nM)**
FASN	13.90 ± 1.99	60.63 ± 11.02**	81.97 ± 19.46**	0.0008/0.0002
ACACA (ACC1)	1.06 ± 0.26	3.72 ± 0.96	4.45 ± 1.09*	0.095/0.049
ACACB (ACC2)	0.49 ± 0.11	1.01 ± 0.18	1.31 ± 0.16*	0.13/0.03
CPT1	1.78 ± 0.25	3.25 ± 0.12*	4.71 ± 0.46**	0.019/0.002

DOI: 10.1530/endoabs.34.P363

P364

Bioinformatic analysis of altered microRNA production in normal adrenal tissue and aldosterone-producing adenoma

Nur Izah Ab Razak[1], Louise Diver[1], Samantha Alvarez-Madrazo[1], Stacy Robertson[1], Martin McBride[1], Paul Stewart[2], John Connell[3], Scott MacKenzie[1] & Eleanor Davies[1]
[1]Division of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK; [2]School of Medicine, University of Leeds, Leeds, UK; [3]School of Medicine, University of Dundee, Dundee, UK.

Hypertension is a common risk factor for cardiovascular disease and up to 15% of hypertensive patients are now known to have primary aldosteronism (PA), where the adrenal glands secrete inappropriately high levels of the steroid hormone aldosterone. Of these cases, almost half are due to the presence of unilateral aldosterone-producing adenoma (APA). Aldosterone is synthesised in the zona glomerulosa of the adrenal cortex, with the final stages of production catalysed by aldosterone synthase, an enzyme encoded by the *CYP11B2* gene. Previous studies showed that *CYP11B2* expression is partly subject to regulation by microRNAs (miRNAs). These are small, single-stranded RNAs 20–25 nucleotides in size that target particular mRNAs, modulating gene expression at the post-transcriptional level. We have now extended our studies to investigate whether microRNA has a role in the aetiology and pathophysiology of APA.
We analysed the microRNA content of four human APA and four normal human adrenal (NA) tissue samples by microarray and then conducted bioinformatic analysis of the resulting data, using software resources including Ingenuity Pathway Analysis (IPA) and DIANA micro-T databases. Of the 78 miRNAs detected above the 500 arbitrary unit (AU) threshold level in one or both tissues, 31 were differentially expressed between NA and APA samples ($P < 0.05$), 18 being more highly expressed in NA samples, and 13 more abundant in APA. IPA analysis confirmed endocrine system disorders as being one of the top three disease states associated with these 31 miRNAs, alongside cancer and cardiovascular. IPA was also used to identify plausible mRNA targets for these 31 miRNAs. A predicted target of particular interest is 3-hydroxy-3-methylglutaryl-CoA-reductase (HMGCR), the mRNA of which is predicted to bind four miRNAs that are each significantly downregulated in APA relative to NA. This gene represents a rate-limiting step in cholesterol production and so may be an important determinant of steroid biosynthesis. Five miRNAs are also predicted to target *CYP11B2* mRNA and may therefore have a direct regulatory influence on aldosterone production.
In summary, miRNA data generated by microarray analysis was analysed bioinformatically in order to identify putative mRNA targets related to functions that may be disrupted in cases of APA, including cell proliferation, apoptosis, and steroidogenesis. These findings will direct future *in vitro* analyses designed to increase our understanding of the aetiology of APA and identify prospective biomarkers for its diagnosis and treatment.

DOI: 10.1530/endoabs.34.P364

P365

^{11}C-metomidate PET–CT in primary hyperaldosteronism: a valuable alternative to AVS

Andrew S Powlson[1], Olympia Koulouri[1], Benjamin G Challis[1], H K Cheow[2], John Buscombe[2], Brendan Koo[2], Morris J Brown[3] & Mark Gurnell[1]
[1]Metabolic Research Laboratories, Wellcome Trust–MRC Institute of Metabolic Science, University of Cambridge, Cambridge, UK; [2]Department of Radiology, Addenbrooke's Hospital, Cambridge, UK; [3]Clinical Pharmacology Unit, Department of Medicine, University of Cambridge, Cambridge, UK.

Although adrenal vein sampling (AVS) remains the gold-standard for distinguishing unilateral and bilateral disease in primary hyperaldosteronism (PHA), it is technically demanding and not always feasible. Metomidate (MTO), a potent inhibitor of CYP11B1 and CYP11B2, can be $C^{11}H_3$-labelled as a PET tracer (^{11}C-MTO), and we have previously shown it to be an alternative to AVS for localising unilateral aldosterone-producing adenomas (APAs) (Burton *et al. JCEM* 2012).

Here, we report a case series in which ^{11}C-MTO PET–CT was superior to AVS in specific settings:

i) AVS technically unsuccessful with failure to cannulate both adrenal veins: ^{11}C-MTO PET–CT accurately localised a unilateral lesion in 12 of 19 cases, with bilateral disease confirmed in 7. Ten of the patients with unilateral PHA have subsequently undergone adrenalectomy, which corrected PHA with normalisation of the aldosterone-to-renin ratio (ARR) in all cases. In several patients with small adenomas that were initially 'missed' on cross-sectional imaging, ^{11}C-MTO PET–CT clearly demonstrated increased tracer uptake corresponding to the site of a small APA as confirmed at adrenalectomy.

ii) Technically adequate AVS, but without lateralisation: in a subgroup of patients with successful bilateral adrenal vein cannulation, but in whom AVS did not meet criteria for lateralisation, ^{11}C-MTO PET–CT showed focal tracer uptake corresponding to a nodule on cross-sectional imaging; six patients have subsequently undergone adrenalectomy, with correction of PHA and normalisation of ARR.

iii) AVS not possible (unable to safely withdraw spironolactone): in patients with refractory severe hypertension, controlled only with spironolactone, ^{11}C-MTO PET–CT successfully localised an APA and facilitated surgery, resulting in correction of PHA (and ARR), with requirement for fewer/no antihypertensive agents post-surgery.

Finally, where multiple nodules co-exist within a single or both glands, ^{11}C-MTO PET–CT often accurately identifies the causative tumour (confirmed by cell culture and genotyping). We speculate this may facilitate non-surgical targeted nodule-specific ablation or selective surgical adenomectomy.

DOI: 10.1530/endoabs.34.P365

P366

Testosterone regulates glucose control in liver and muscle of Tfm mice as a mechanism to improve type 2 diabetes

Daniel Kelly[1], Samia Akhtar[1], Vakkat Muraleedharan[1,2], Donna Sellers[3], Johnathan Brooke[1], David McLaren[1] & Hugh Jones[1,2]
[1]University of Sheffield, Sheffield, UK; [2]Barnsley Hospital NHS Foundation Trust, Barnsley, UK; [3]Sheffield Hallam University, Sheffield, UK.

Introduction

There is a strong association between testosterone deficiency and the incidence of type 2 diabetes (T2D) in men. Physiological testosterone replacement (TRT) improves insulin resistance and glycaemic control in hypogonadal men. The mechanism by which testosterone mediates these beneficial actions are unknown but may be due to an effect on major metabolically active tissues such as liver and muscle. This study investigates the expression of key regulatory targets of glucose control in liver and muscle tissue of the testicular feminised mouse (Tfm) which exhibit non-functional androgen receptors and low circulating testosterone.

Methods

Tfm mice fed a high-cholesterol diet ad libitum for 28 weeks received either physiological testosterone replacement or placebo and were compared to WT littermates (WT). Liver and muscle tissue was collected and relative concentrations of mRNA and protein were analysed by qPCR and western blotting for expression of hexokinase 2 and 4 (Hk2 and Hk4), glucose transporter 4 (Glut4), phosphofructokinase (Pfk), insulin receptor substrate 1 (Irs1), MAPKK1 (Map2k), carbohydrate regulatory element binding protein (Chrebp), glucose-6-phosphate 1-dehydrogenase X (G6pdx), and glycogen synthase (Gys1).

Results

There was a significant decrease in the relative mRNA expression of Hk4, Pfk and Map2k in liver and Glut4, Hk2, Pfk and Map2k in muscle of Tfm mice compared to WT. G6pdx was increased in Tfm liver. TRT increased hepatic Hk4 mRNA and decreased G6pdx. TRT had no effect on muscle mRNA expression. Western blotting confirmed reduced muscle protein expression of Hk2 and Glut4, and decreased hepatic HK4, PFK in Tfm mice compared to WT with TRT increasing hepatic Hk4. This suggests both AR-independent and dependent mechanisms. No differences were observed between animal groups for the expression of Irs1, Chrebp, and Gys1.

Conclusion

Testosterone differentially regulates the expression of key targets involved in glucose homeostasis in liver and muscle as a mechanism to potentially improve T2D.

DOI: 10.1530/endoabs.34.P366

P367

The endocrine response to severe trauma: the Steroids and Immunity from injury to Rehabilitation (SIR) study

Mark Foster[1,3], Angela Taylor[1], Neil Hill[3,5], Rob Staruch[2,3], Donna O'Neil[1], Julian Bion[1,2], Stephen Young[1], Mark Midwinter[1,3], Janet Lord[1] & Wiebka Arlt[1]
[1]University Of Birmingham, Birmingham, UK; [2]University Hospital Birmingham, Birmingham, UK; [3]Royal Centre Of Defence Medicine, Birmingham, UK; [4]Institute Of Naval Medicine, Gosport, Hants, UK; [5]Imperial College London, W12 0NN, London, UK.

There are 1.21 million deaths from road traffic accidents worldwide. In Afghanistan, there have been 2005 battle injuries over 10 years. Advances in military trauma care have improved survival, resulting in more severely injured individuals entering the trauma care pathway. Improved understanding of endocrine-immune changes after severe trauma may facilitate novel interventions to improve outcomes. We prospectively recruited 102 severely injured patients at the Queen Elizabeth Hospital Birmingham; 52 military and 50 civilian patients with a mean injury severity score of 27.2 ± 13.9. Blood and 24-h urine was collected at baseline (injury <24 h) and after 1–6 weeks and 3–6 months. Measurements included serum and urinary steroids by mass spectrometry, neutrophil phagocytic activity, urinary nitrogen excretion and muscle thickness by ultrasound; comparisons were made to 30 healthy controls and 100 military controls under combat stress. Results demonstrated a significant increase in serum cortisol, peaking at 2 weeks, with an increased cortisol:cortisone ratio returning to normal after 4 weeks. Both DHEA and DHEAS were significantly down-regulated ($P<0.0001$) and while DHEA returned to normal after 2 months, DHEAS remained very low throughout the 6 months of follow-up. Serum testosterone was initially completely suppressed ($P<0.0001$) but normalised after week 4. Neutrophil phagocytosis was significantly impaired and returned to normal only after 3 months. Urinary protein loss and muscle mass followed a U-shaped curve with an initial steep decrease followed by recovery to normal at months 2 and 3 post-injury, respectively. In conclusion, the acute response to severe injury comprises increased glucocorticoid activation and down-regulation of adrenal and gonadal androgens. The resurgence of testosterone 4 weeks post-injury initiates an anabolic recovery phase, however, neutrophil phagocytosis shows only delayed recovery and serum DHEAS remained suppressed until 6 months post-injury. Delineation of whether the endocrine changes are beneficial or adverse will determine the potential for future intervention studies.

DOI: 10.1530/endoabs.34.P367

P368

Glucocorticoid activation in muscle by 11β-hydroxysteroid dehydrogenase type 1: contributions to inflammatory muscle wasting

Rowan Hardy[1,4], Gareth Lavery[1], Mark Pierson[4], Craig Doug[1], Andrew Filer[4], Christopher Buckley[4], Janet Lord[4], Paul Stewart[3], Mark Cooper[2] & Karim Raza[4]
[1]Clinical and Experimental Medicine 2nd floor IBR, The University of Birmingham, Birmingham, West Midlands, UK; [2]The University of Sydney, Concord Clinical School, Sydney, New South Wales, Australia; [3]Faculty of Medicine and Health, School of Medicine Leeds, The University of Leeds LS2 9NL, UK; [4]School of Immunity and Infection, 2nd floor IBR, The University of Birmingham, Birmingham, West Midlands, UK.

Muscle wasting remains a significant complication in patients with inflammatory disease where it contributes to disability, risk of falls and early mortality. Interestingly, muscle wasting in patients with glucocorticoid excess mirrors that observed in patients with inflammatory disease. We have previously reported that the glucocorticoid activating enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is potently up-regulated within mesenchymal derived cell populations in response to pro-inflammatory stimuli.

Consequently, we hypothesise that muscle wasting in patients with inflammatory disease is driven by elevated glucocorticoid activation in mesenchymal derived myocytes. In this study we have collected biopsies of quadriceps from 14 patients with osteoarthritis. 11β-HSD1 expression and activity in muscle was examined by real-time RT PCR, immunohistochemistry and tritiated steroid conversion assays. *In vitro* regulation of 11β-HSD1 by the pro-inflammatory cytokines TNFα and IL1β (10 ng/ml) was assessed in primary myocyte culture.

Positive 11β-HSD1 mRNA expression was identified in muscle (Avg $\Delta Ct = 16.6 \pm 0.75$), with enzyme expression observed ubiquitously throughout the tissue. This was supported by significant oxoreductase activity (14 ± 5 fmol/mg tissue per hr). In primary cultures of myocytes, 11β-HSD1 was potently up-regulated at 24 h by the cytokines TNFα and IL1β (7.8 ± 0.9 and 18.2 ± 2.1-fold respectively; $P < 0.05$). This effect was abrogated by the NFκB inhibitor parthenolide (1 μM). 11β-HSD1 mRNA expression in muscle was shown to positively correlate with markers of inflammation (IL6, $R^2 = 0.62$; $P < 0.05$) and atrophy (FOXO-1, $R^2 = 0.36$; $P < 0.05$).

These findings demonstrate that 11β-HSD1 is actively expressed within human muscle and primary myocytes, where it is positively regulated by pro-inflammatory cytokines and correlates with markers of muscle inflammation and atrophy. Consequently, these data support a role for glucocorticoid activation by 11β-HSD1 in driving inflammatory muscle wasting.

DOI: 10.1530/endoabs.34.P368

P369

Abstract Withdrawn.

DOI: 10.1530/endoabs.34.P369

P370

A very late and unusual presentation of congenital adrenal hyperplasia

Ahmad Abou-Saleh, Dennis Barnes & Masud Haq
Maidstone and Tunbridge Wells NHS Trust, Tunbridge Wells, Kent, UK.

A 71-year-old gentleman was referred for investigation of bilateral enlarged adrenals first discovered after presenting with subacute bowel obstruction. A CT scan of his abdomen revealed the left adrenal was 8×5 cm, the right 3 cm. No other abnormalities were detected.

The patient was otherwise well. No other medical history was volunteered. On examination, he was of short stature (height 1.47 m) with a normal pattern of pubic and axillary hair. Blood pressure was 140/86 mmHg. Given his age, metastatic involvement of the adrenals was the primary diagnosis. Initial investigations revealed a low 0900 h cortisol. A subsequent short Synacthen test confirmed hypoadrenalism: baseline cortisol, 149 nmol/l; 30 min, 236 nmol/l; 60 min, 277 nmol/l; ACTH was 36 ng/l (<46; 0900 h). Urinary catecholamines were consistently normal. The patient was commenced on hydrocortisone treatment (15 mg at 0700 h and 10 mg at 1600 h) and proceeded to have further investigations which revealed normal fasting gut hormones, neurone specific enolase, and mIBG scan. He proceeded to have a CT guided adrenal biopsy which demonstrated features consistent with a primary adrenocortical tumour.

On further review, the patient had been treated for hypospadias as a teenager. He reported good libido, but suffered with erectile dysfunction and had never fathered a child. Examination of his external genitalia revealed an under-developed penis but no palpable testes. Gonadotrophins were elevated (LH 25.2 IU/l, FSH 32.4 IU/l), 0900 h testosterone was 13.2 nmol, but 17-hydroxy-progesterone was significantly elevated at 37.6. Renin activity was normal. His karyotype was 46XX consistent with late onset congenital adrenal hyperplasia (CAH). His bilateral adrenals were therefore consistent with hyperplasia of adrenal rest cells. The patient responded favourably to steroid replacement but was not informed of his karyoptype as he was happily married for 50 years.

This case illustrates a very unusual late presentation of CAH.

DOI: 10.1530/endoabs.34.P370

P371

Enhanced expression of hepatic inflammatory markers in 11β-hydroxysteroid dehydrogenase type 1 knockout mice fed a steatogenic diet

Dean Larner[1], Stuart Morgan[1], Laura Gathercole[1], Maryam Nasiri[1], Philip Guest[1], Matthew Chapman[1], Jeremy Tomlinson[1], Paul Stewart[2] & Gareth Lavery[1]
[1]University of Birmingham, Birmingham, UK; [2]University of Leeds, Leeds, UK.

Non-alcoholic fatty liver disease (NAFLD) is characterised by intra-hepatocyte lipid accumulation. Simple steatosis, which is a reversible condition, can progress to non-alcoholic steatohepatitis (NASH), cirrhosis and eventually hepatocellular carcinoma. The aetiology of NAFLD is not fully understood and it is suggested that glucocorticoid reactivation through the activity of the 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) enzyme may promote hepatic lipid accumulation and contribute to the potential to develop steatosis. To test this, 11β-HSD1KO and C57BL/6 control mice ($n = 11$–13) were fed the American life style induced obesity syndrome (ALIOS) diet, known to induce NAFLD, for 16 weeks. Mice were weighed weekly and glucose and insulin tolerance tests (GTT and ITT respectively) performed. We found that there were no differences between bodyweights or fasting blood-glucose concentrations, with GTTs and ITTs showing no differences between 11β-HSD1KO and controls. There was no difference between liver weights of WT and KOs. To assess hepatic histopathology, H&E stained sections were blind scored for the degree of steatosis (0–3, zero being absent, three being severe). In control livers, an average score of 1.8 was seen, which was elevated to 2.3 in 11β-HSD1KO livers. Finally, we have expression profiled our cohorts and did not observe any changes in the expression of genes associated with metabolic processes or reactive oxygen species defence. However, as the ALIOS diet can induce hepatic inflammation, we assessed the expression of a series of inflammatory markers and had highly significant increases in the mRNA of Tnfa ($P < 0.001$), Col1a1 ($P < 0.01$) and Fsp ($P < 0.001$) in 11β-HSD1KO mice compared to controls. While the ablation of 11β-HSD1 does not ameliorate steatosis in mice, it does evoke increased expression of inflammatory response genes that could exacerbate the progression of simple steatosis to NASH. These data highlight the important role 11β-HSD1 may play in restricting inflammation associated with the steatotic liver.

DOI: 10.1530/endoabs.34.P371

P372

Cushing's syndrome, missed in pregnancy

Koshy Jacob[1] & Pushpa Jinadev[2]
[1]Pilgrim Hospital, United Lincolnshire NHS Trust, Boston, UK;
[2]Manchester Royal Infirmary, Manchester, UK.

Mrs XY, 28 years. primigravida was seen for PV bleed. Ultrasonogram confirmed live intrauterine foetus of 8 weeks gestation with no obvious cause for PV bleeding. An OGTT at 21 weeks suggested gestational diabetes. She was referred to Diabetes Nurse led clinic and treated with metformin. Hypertension was recorded and controlled with labetalol and amlodipine. Mrs XY had recurrent admissions for PV bleeds and some polycythaemia. She was seen by the haematologist and a diagnosis of transient polycythaemia was made. Mrs XY then presented with pre-eclampsia at 36 weeks resulting in emergency LSCS and healthy baby. Six months *postpartum* she was referred to endocrine clinic for persisting hypertension and facial swelling. O/e she had proximal myopathy, easy bruising and purplish abdominal striae. BP was controlled on labetalol 200 mg bid, BMI: 21.2, HbA1c 31 mmol/mol, 24 h. urinary catecholamine's and androgen profile were normal. 24 h urinary free cortisol was elevated with 1 mg overnight dexamethasone suppression test and a low dose dexamethasone suppression test not suppressing. 0900 h ACTH levels were suppressed and a diagnosis of ACTH independent Cushing's syndrome was made. CT scans confirmed right adrenal adenoma. Ketoconazole was started for inhibition of steroidogenesis. Mrs XY underwent laparoscopic right adrenalectomy with histology confirming adrenocortical adenoma. Post-surgery her blood pressure normalised.

Discussion

Cushing's syndrome is a rare diagnosis in pregnancy which can result in significant maternal and foetal morbidity. Adrenal adenomas comprise most of Cushing's seen in pregnancy as was in our patient. The signs and symptoms of hypercortisolemia overlap with normal pregnancy making it difficult to diagnose. In normal pregnancy the diurnal rhythm of cortisol secretion is preserved but there is increase in ACTH, total cortisol as well as free plasma cortisol making biochemical diagnosis difficult. UFC >1 times upper limit of normal in 1st trimester and UFC >3 times upper limit of normal in 2nd or 3rd trimester along with demonstration of loss of diurnal rhythm preferably by salivary cortisol can aid diagnosis of Cushing's syndrome. A high index of suspicion is necessary to consider Cushing's in pregnancy especially if there is excessive weight gain, and a combination of gestational diabetes and hypertension.

DOI: 10.1530/endoabs.34.P372

P373

Dutasteride and 5α-reductase type 1 activity: for androgens only?

Charles West, Royce Vincent & Norman Taylor
King's College Hospital, London, UK.

Background

Three 5α-reductase (5α-R1-3) isoenzymes are described. In humans, 5α-R2 deficiency (5α-R2D) causes pseudohermaphroditism and 5α-R3 neurological defects. The role of 5α-R1 is not clear. Urine steroid profiling (USP) by GC–MS can compare 5α-reduction activity based on excretion of 5α- and 5β-reduced metabolite ratios. Finasteride predominantly inhibits 5α-R2. USP in these patients shows concordance with 5α-R2D. Dutasteride is a dual inhibitor of 5α-R1 and 5α-R2. We compared USP data on patients using finasteride and dutasteride against 5α-R2D to uncover the role of 5α-R1 in generating urinary 5α-reduced metabolites.

Methods

We completed intercomparison of 5α/5β-reduced urinary steroid ratios by USP in genetically confirmed 5α-R2D ($n=28$), finasteride ($n=6$) and dutasteride ($n=2$) treatment with controls ($n=36$). The ratios studied were: androgens, androsterone (A)/aetiocholanolone and 11β-OH androsterone (11OHA)/11β-OH aetiocholanolone; and corticosteroids, allo-tetrahydrocorticosterone/tetrahydrocorticosterone and allo-tetrahydrocortisol/tetrahydrocortisol.

Results

Study groups showed significant decrease of all ratios relative to controls. Values (ranges) for the four ratios in the sequence listed above were: 5α-R2D, 0.10–10.0; 0.17–20.7; 0.10–1.67; 0.00–0.10, finasteride, 0.07–0.28; 0.20–2.31; 0.07–3.42; 0.00–0.09, dutasteride, 0.02–0.03; 0.07–0.29; 0.55–0.61; 0.00–0.01. Thus there was no difference for corticosteroids between the groups, but the dutasteride patients showed significantly lower androgen metabolite ratios compared to 5α-R2D.

Conclusions

These findings support the expectation that 5α-reduction of corticosteroids is almost exclusively dependent on 5α-R2 activity, this explains why these ratios are the most diagnostic for 5α-R2D. Lower excretion of A and 11OHA in the urine with dutasteride use indicates 5α-R1 has a significant role in androgen catabolism. This may be true for 5α-reduction of testosterone and dihydrotestosterone and explain the lower diagnostic sensitivity of their ratio in serum in 5α-R2D. There is evidence of derangement of androgen metabolism in animal 5α-R1 knock-out studies, thus potential for currently unidentified pathology if 5α-R1 deficiency exists in humans.

DOI: 10.1530/endoabs.34.P373

P374

Differential impact of PAPS synthases on human sulfation pathways

Jonathan W Mueller[1], Jan Idkowiak[1], Philip J House[1], Joane McNelis[1,3], Ian I Rose[1], Johannes van den Boom[2], Florian Schlereth[1], Vivek Dhir[1] & Wiebke Arlt[1]
[1]University of Birmingham, West Midlands, UK; [2]University of Duisburg-Essen, Essen, Germany; [3]University of California, San Diego, California, USA.

Mutations in the gene for 3′-phospho-adenosine-5′-phosphosulfate synthase 2 (PAPSS2) are linked to bone and cartilage mal-formation. More recently, we could identify *PAPSS2*-mutations as mono-genetic cause for androgen excess in women due to apparent SULT2A1 deficiency, the enzyme responsible for sulfation of the testosterone precursor DHEA that relies on PAPS provision by PAPS synthases. The only human orthologue, PAPSS1, is expressed in the affected tissues at comparable levels to PAPSS2, suggesting non-overlapping functionality of the two proteins. Here, we characterised specific activity of the sulfurylase and APS-kinase activities by coupled enzyme assays as well as ligand-binding affinity by fluorescence titrations using fluorescently-labelled APS for both PAPS synthases and found remarkable similarity. We then characterised expression levels of PAPS synthases by RT-PCR in hepatic and adrenal cell lines. From this analysis, we chose the adrenal cell line NCI-H295R1 for siRNA-mediated knockdown of PAPSS1/S2 as these two genes were expressed at similar levels. siRNA-knockdown was confirmed by real-time PCR and western blot for PAPSS1, PAPSS2 and SULT2A1. Functionally, SULT2A1 knockdown reduced DHEA-sulfation to $19\pm6\%$ of the control reaction and a knockdown of PAPSS2 resulted in $30\pm5\%$ conversion, while a PAPSS1-knockdown did not reduce DHEA-sulfation. Next, we analysed the influence of subcellular localisation of PAPS synthases on their ability to support DHEA-sulfation by co-expressing nuclear and cytoplasmic variants of PAPSS1/S2 in HEK293 cells with SULT2A1. While WT and nuclear PAPSS1/S2 proteins supported DHEA-sulfation to a similar extent, cytoplasmic PAPSS2 caused significantly higher SULT2A activity (160%) than cytoplasmic PAPSS1. Finally, we conducted a proteomics screen for PAPSS2-interaction partners in HEK293-FlpIn cells expressing PAPSS2-Strep-HA followed by pull-down and LC-MS/MS analysis. Proteasomal subunits are significantly enriched in our dataset, suggesting active protein turn-over of PAPSS2 involving the proteasomal machinery. Whether this is responsible for the functional differences between PAPS synthases remains to be elucidated in the future.

DOI: 10.1530/endoabs.34.P374

P375

Increased lipopolysaccharide-induced neutrophilia in mice lacking the glucocorticoid receptor in bronchial epithelial (Clara) cells

Louise Kearney[1], Julie Gibbs[2], Stuart Farrow[3], David Ray[2] & Andrew Loudon[1]
[1]Faculty of Life Sciences, University of Manchester, Manchester, UK; [2]Faculty of Medical and Human Sciences, Institute of Human Development, University of Manchester, Manchester, UK; [3]Respiratory Therapy Area, Medicines Research Centre, GlaxoSmithKline plc, Stevenage, UK.

One in five people in the UK is affected by lung disease, along with millions more worldwide. Glucocorticoids represent the most utilized anti-inflammatory therapy for the treatment of pulmonary inflammation, however a subset of patients exist which do not respond to therapeutically relevant doses.

The non-ciliated bronchial epithelial (Clara) cells have been identified as key mediators of the pulmonary inflammatory response. The glucocorticoid receptor (GR) is expressed ubiquitously throughout the lung, including within the Clara cells. To test whether the effectiveness of glucocorticoid therapy is linked to signalling through these cells we bred the GR^{flox} mouse (Schütz lab, DKFZ, via the European Mutant Mouse Archive) with $CCSP^{icre}$ mice (DeMayo lab, Baylor College of Medicine, Texas) to generate a novel transgenic mouse line with targeted deletion of GR in the Clara cell.

Aerosolised bacterial endotoxin (lipopolysaccharide, LPS) was utilised as a tissue-specific stimulus to provoke a localised neutrophilic inflammatory response within the lung. Mice lacking GR in Clara cells (GR^{flox}, $CCSP^{icre}+ve$) exhibit a two fold increase in neutrophil recruitment to the lung compared to $CCSP^{icre}$–ve littermates. However, in line with the control littermates, this neutrophil infiltration is ameliorated by pre-treatment with intraperitoneal injection of dexamethasone (1 mg/kg).

These results illustrate the importance of the Clara cell GR in mediating the anti-inflammatory effects of endogenous glucocorticoids, but also implicate additional GR expressing cell types within the lung in modulating dexamethasone-induced repression of the pulmonary inflammatory response.

DOI: 10.1530/endoabs.34.P375

P376

Blocking local glucocorticoid activation improves skin thinning and impaired healing in Cushingoid mice

Ana Tiganescu, Yoshikazu Uchida, Peter Elias & Walter Holleran
University of California San Francisco (NCIRE), San Francisco, California, USA.

Cushing's disease presents with multiple symptoms of systemic glucocorticoid (GC) excess including increased skin thinning and poor wound healing (WH). Local GC concentrations are regulated by 11β-hydroxysteroid dehydrogenase (11β-HSD) isozymes converting inactive cortisone/11-dehydrocorticosterone to cortisol/corticosterone (11β-HSD1) or *vice versa* (11β-HSD2). We previously demonstrated elevated 11β-HSD1 activity during early WH, hypothesized to antagonize wound repair.

We examined the effects of systemic GC excess (or vehicle) in female SKH1-hr mice which developed Cushingoid features and suppressed endogenous serum corticosterone vs vehicle (CORT, 5.5 vs 18.5 ng/ml, $P<0.01$) over 5 weeks CORT therapy (100 μg/ml in drinking water). Mice were treated bi-daily with 30 mM (200 μg) topical carbenoxolone (CBX, 11β-HSD inhibitor) or vehicle, 1 week prior to and post-wounding (by double 5 mm dorsal biopsy).

11β-HSD1 activity was 42% higher in unwounded CORT skin (3.1 vs 2.2 pmol/h, $P<0.05$) and inhibited >60% by CBX. CORT-induced epidermal thinning was 35% lower with CBX (10.1 vs 15.5 μm, $P<0.01$) and whilst CORT-treated skin required more desquamation (7.6 vs 4.9 tapes, $P<0.001$) to induce barrier disruption (trans-epidermal water loss >30 g/h per m^2) this was 67% normalized by CBX ($P<0.05$). Supporting studies in primary human keratinocyte revealed 11β-HSD1 activity increased seven fold during differentiation ($P<0.001$). Moreover, cortisol treatment induced differentiation (loricrin) and suppressed proliferation (keratin-10) marker expression (normalized to β-actin by Western Blot); effects reversed by GC receptor (RU486) co-treatment. During WH, 11β-HSD1 activity increased to 5 pmol/h in vehicle and CORT-treated mice ($P<0.001$). However, whilst the former declined 25% by day 9 ($P<0.05$), the latter remained elevated. Furthermore, although day 6 wounds were 50–80% larger in all CORT mice (vs vehicle, $P<0.01$), subsequent healing was observed only with CBX co-treatment (34% by day 9, $P<0.01$).

Although CBX treatment did not affect any of the above parameters in young, healthy mice, topical 11β-HSD1 inhibitors may improve adverse dermatological consequences of systemic GC excess.

DOI: 10.1530/endoabs.34.P376

P377

Increased 11β-hydroxysteroid dehydrogenase type 1 activity in UVB-irradiated mice

Ana Tiganescu, Yoshikazu Uchida, Peter Elias & Walter Holleran
University of California San Francisco (NCIRE), San Francisco, California, USA.

UVB exposure induces skin damage including dermal atrophy, telangiectasia, fragility and poor wound healing; symptoms also attributable to glucocorticoid (GC) excess (e.g. Cushing's syndrome). In peripheral tissues including skin, GC availability is regulated by 11β-hydroxysteroid dehydrogenase (11β-HSD) types 1/2 which respectively activate/deactivate cortisol (and rodent corticosterone) from/to cortisone (and rodent 11-dehydrocorticosterone). Although we previously demonstrated increased 11β-HSD1 levels in photo-exposed vs photo-protected human skin, direct regulation of 11β-HSD1 expression by UVB in *vivo* remains unexplored.

We irradiated female SKH1-hr mice with 0, 50, 100, 200 and 400 mJ UVB/cm^2 and collected skin tissue at 0, 1, 3 and 7 days (d0 etc.) post-exposure. At 400 mJ, 11β-HSD1 mRNA expression (measured by qPCR normalized to 18S rRNA) increased 2.7-fold at d1 ($^{P}<0.01$), remaining high but more variable at subsequent timepoints. Hexose-6-phosphate dehydrogenase (11β-HSD1 cofactor-supplying enzyme) mRNA expression also increased 2.5-fold at d1 ($P<0.05$) returning to non-irradiated levels by d7. Conversely, GC receptor mRNA was 31% lower at d3 ($P<0.05$). 11β-HSD2 is not expressed in murine skin.

At 400 mJ, 11β-HSD1 activity (determined by incubation with 100 nM tritiated 11-dehydrocorticosterone) was 76% greater than d0 only at d3 (3.6 vs 2.1 pmol/h, $P<0.05$) and was also increased by 100 and 200 mJ (40%, $P<0.05$ and 50%, $P<0.001$ respectively). Localization studies (by immunofluorescence) revealed 11β-HSD1-positive staining in hyperproliferative epidermis vs non-irradiated skin at 100 and 200 mJ (81.2 and 77.4 vs 21.2 μm, $P<0.001$) with increased stain intensity but reduced hyperproliferation at 400 mJ (41 μm, $P<0.001$). Dermal 11β-HSD1 expression was unaffected by UVB exposure.

Our findings suggest increased 11β-HSD1 activity following UVB exposure *in vivo* may exacerbate UV-induced skin damage by increasing local GC availability. Topical 11β-HSD1 inhibitors may minimize adverse consequences of UVB exposure.

DOI: 10.1530/endoabs.34.P377

P378

The Prevention of Adrenal Crisis in Stress (PACS) study: serum cortisol during elective surgery and acute trauma in comparison to stress dose hydrocortisone in adrenal insufficiency

Angela Taylor[1], Niki Karavitaki[2], Mark Foster[3], Sibylle Meier[2], Donna O'Neil[1], John Komninos[2], Dimitra Vassiliadi[1], Christopher Mowatt[1], Janet Lord[3], John Wass[2] & Wiebke Arlt[1]
[1]School of Clinical and Experimental Medicine, Centre for Endocrinology, Diabetes and Metabolism (CEDAM), University of Birmingham, Birmingham, UK; [2]Oxford Centre for Diabetes, Endocrinology and Metabolism, Churchill Hospital, Oxford, UK; [3]NIHR SRMRC, School of Immunity and Infection, University of Birmingham, Birmingham, UK.

Patients with adrenal insufficiency (AI) require adjustment of hydrocortisone (HC) dose to avoid life-threatening adrenal crisis during illness, surgery and trauma. However, current dose recommendations are based on empirical grounds only and choice of dose and administration modes vary considerably. We designed the PACS study to compare cortisol levels achieved by currently recommended HC stress doses to those in i) healthy controls ($n=85$, 21–70 years), ii) military controls under combat stress conditions ($n=105$, 20–40 years), iii) patients undergoing elective moderate or major surgery ($n=22$, age range 21–60 years), and (iv) patients admitted after acute trauma ($n=85$, 30–60 years). Ten patients with autoimmune primary AI (40–64 years) underwent frequent serum sampling after 200 mg HC/24 h in four different administration modes: 50 mg orally every 6 h, 50 mg i.m. every 6 h, 50 mg i.v. every 6 h, or 200 mg HC per continuous i.v. infusion. Serum cortisol was measured by tandem mass spectrometry (Waters Xevo/Acquity uPLC). Cortisol levels during moderate (median 431, range 249–570 nmol/l) and major elective surgery (611, 165–1102 nmol/l) peaked between 2 and 4 h after anaesthesia induction. C_{max} values for acute trauma patients were 433 (106–685) nmol/l. C_{max} after HC administration via any administration mode had median values ranging from 836 to 1440 nmol/l. However, nadir cortisol levels during intermittent bolus application of HC decreased to C_{min} 277 (64–398), 289 (148–458), and 173 (118–375) nmol/l 6 h after administration of oral, i.m. and i.v. HC bolus, respectively. By contrast, continuous infusion of HC yielded steady-state cortisol concentrations after 1 h with a median of 836 (range 661–1073) nmol/l. HC dose cover during surgery, trauma and major illness in patients with AI should be provided by continuous i.v. infusion of 200 mg HC/24 h, following an initial HC bolus administered at admission or anaesthesia induction.

DOI: 10.1530/endoabs.34.P378

P379

Abstract withdrawn.

DOI: 10.1530/endoabs.34.P379

Thyroid

P380

Suspected DVT after thyrotoxicosis

Rakhi Kakad, Munirul Haque & John Milles
Good Hope Hospital, Sutton Coldfield, UK.

We present the case of a young man who had been known to the endocrine department with difficult to control hyperthyroidism and atrial fibrillation. After a period of treatment with carbimazole, during which he missed several appointments, he was referred for radioactive iodine therapy, which was administered in January 2013. He failed to attend for follow-up appointments and continued to take carbimazole.

He was subsequently referred to the acute medicine department, in May 2013 with bilateral leg pain and suspected DVT. Both legs were swollen, left more than right. He had a foot drop on the left side. Investigations confirmed raised CK at 1295, rising to 12 192. MRI of the left leg suggested myositis but could not exclude acute compartment syndrome.

He underwent left lower limb fasciotomy the following day to relieve pressure in the anterior compartment. Muscle biopsy taken at surgery showed normal architecture, suggesting that permanent damage had been avoided.

He was noted to have raised TSH at 52.8 (NR 0.4 – 4.9) and fT_4 <5.2 (NR 9–19). His carbimazole was stopped and he was started on levothyroxine during his admission. His thyroid status is now normal, though he has a persistent unilateral foot drop.

Acute compartment syndrome has previously been described in primary hypothyroidism and in primary hypothyroidism with co-morbidities including Addison's, statin use and post-surgery. One report links induced hypothyroidism with acute compartment syndrome with co-existing diabetic neuropathy. Compartment syndrome has not previously been reported in induced hypothyroidism without co-morbidities, and this case highlights the link between thyroid function and acute compartment syndrome, and a particular concern with induced hypothyroidism. The case also raises questions about the use of radioiodine where follow-up is unpredictable.

DOI: 10.1530/endoabs.34.P380

P381

Post-operative thyroiditis: an under recognised clinical phenomenon

Rashmi Manjunatha[1], D H Markham[2] & Rajni Mahto[1]
[1]Department of Diabetes and Endocrinology, Warwick Hospital, Coventry, UK; [2]Department of ENT, Warwick Hospital, Coventry, UK.

A 34-year-old lady with previous renal stones, constipation and well controlled bipolar mood disorder, on lithium for 13 years, was referred with a high calcium of 2.75 mmol/l (normal range: 2.10–2.58 mmol/l) and raised parathyroid hormone of 9.1 pmol/l (normal range: 1.1–4.2 pmol/l). Urine calcium excretion and TFT were normal.

The biochemistry was consistent with primary hyperparathyroidism. A workup for possible underlying MEN syndrome came back as negative. An ultrasound scan of the parathyroid showed three tiny soft tissue hypoechoic nodules inferior to the lower pole of the left lobe of thyroid, raising a suspicion of parathyroid adenomas. However, a sestamibi scan was unable to localise any parathyroid adenoma. She underwent a bilateral neck exploration and four gland parathyroidectomy and thymectomy as a fifth parathyroid gland was felt to be embedded within the thymus. Four days post operatively, she was admitted with confusion, tachycardia and carpo-pedal spasm. She was hypocalcemic (Ca:1.93 mmol/l) which was treated with Sandocal and alfacalcidol. TFT revealed a thyrotoxic picture with free T_4 >100 pmol/l, free T_3 of 50 pmol/l and TSH <0.02 mU/l. This raised a suspicion of possible surgery induced thyroiditis caused by handling of the thyroid during surgery. She was treated with propylthiouracil, propranolol along with antibiotics to cover for possible surgical site infection. TFTs improved dramatically and normalized in about 3 weeks. Propylthiouracil dose was tapered and stopped within few weeks.

Although post-operative thyroiditis is well described, it is under recognised. Manipulation of the thyroid gland either during neck exploratory surgery or repeated palpation can result in inflammation of the thyroid gland. Hyperthyroidism is usually transient due to leakage of the preformed thyroid hormone in blood. Careful attention to the thyroid test should be paid in the post-operative period following surgical procedures in which thyroid gland has been manipulated.

DOI: 10.1530/endoabs.34.P381

P382

Prevalence and clinical associations of calcium-sensing receptor autoantibodies in finnish APECED patients

Mahmoud Habibullah[1], Elizabeth H Kemp[1], Nicolas Kluger[2], Annamari Ranki[2], Harpreet K Sandhu[1], Kai J E Krohn[3] & Anthony P Weetman[1]
[1]University of Sheffield, Sheffield, UK; [2]University of Helsinki and Helsinki University Central Hospital, Helsinki, Finland; [3]Clincal Research Institute, Helsinki, Finland.

Context

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is caused by mutations in the autoimmune regulator (AIRE) gene and is characterised by the presence of chronic mucocutaneous candidiasis, hypoparathyroidism and Addison's disease. Patients typically display organ-specific autoantibodies which correlate with a particular clinical manifestation. Previous studies have identified the parathyroid-expressed calcium-sensing receptor (CaSR) as an autoantibody target in APECED. However, it is unclear if antibodies against the CaSR correlate with APECED-associated hypoparathyroidism.

Objective

To identify associations between the presence of CaSR antibodies and the disease manifestations and demographic characteristics of APECED patients.

Design, subjects and methods

This was a case–control study including 44 APECED patients and 38 healthy control subjects. Antibodies against the CaSR were detected using immunoprecipitation assays.

Results

CaSR antibodies were detected in 16 out of 44 (36%) APECED patients and in none of 23 (0%) healthy control subjects ($P \leq 0.0001$). No statistically significant associations were found between the presence of CaSR antibodies and the disease manifestations of APECED including hypoparathyroidism (P values were >0.05). The detection of CaSR antibodies had a specificity of 83%, and a sensitivity of 39% for the diagnosis of hypoparathyroidism. There were no significant associations between the presence of CaSR antibodies and either sex, age or disease presentation age (P values were >0.05). However, a significant association between a shorter APECED duration (<10 years) and positivity for CaSR antibodies was noted ($P = 0.019$).

Conclusion

CaSR antibodies were not found to be a specific or sensitive marker for hypoparathyroidism in APECED. Further investigations are required to determine the exact role of the autoimmune response against the CaSR in the pathogenesis of this autoimmune syndrome.

DOI: 10.1530/endoabs.34.P382

P383

Incidence of Sunitinib induced thyroid dysfunction in renal cell carcinoma: a pilot retrospective audit

Sviatlana Zhyzhneuskaya, Leanna Erete, Justyna Czechowska, Wendy Anderson, Alison Humphreys & Sath Nag
James Cook University Hospital, Middlesbrough, UK.

Introduction
TKI are an emerging group of anti-growth factor agents used in the treatment of solid cancers. Treatment is associated with thyroid dysfunction. Sunitinib is licensed for the treatment of metastatic RCC. Our objective was to determine the incidence of Sunitinib induced thyroid dysfunction and its management in patients with renal RCC.
Methods
Retrospective case note analysis of patients started on Sunitinib for metastatic RCC between January 2010 and December 2012 at the Oncology Unit in JCUH.
Results
31 patients were started on Sunitinib between 2010 and 2012.One patient had pre-existing primary hypothyroidism and was excluded from analysis. Baseline TFTs were done in 93% of patients. The majority of patients ($n=26$) were euthyroid pre-treatment; at baseline, three patients (10%) had subclinical hypothyroidism and one patient (3.3%) had subclinical hyperthyroidism. Mean duration of follow-up was 53.33 weeks. Mean interval between starting Sunitinib and 1st TFT check – 3.3 weeks (range 2–12 weeks, s.d. 2.28). Mean interval to developing abnormal TSH was 9.3 weeks (range 2–42 weeks, s.d. 11.58). Primary hypothyroidism in this cohort developed at 27.7 weeks (range 4–46 weeks). The mean time to commencing levothyroxine (LT_4) therapy was 55.5 weeks (range 21–105 weeks). Sunitinib induced hypothyroidism developed in six patients (20%) whilst subclinical hypothyroidism developed in two patients (6.6%). 21 patients (70%) were biochemically euthyroid. Thyroid status of one patient with baseline subclinical hyperthyroidism remained unchanged. Four patients (13.3%) developed transient subclinical hypothyroidism. Mean TSH level at the start of LT_4 therapy was 55.7 mIU/l (range 19.43–107.24) and mean free T_4 was 8.4 pmol/l (range 3.5–11.8). LT_4 was commenced at a mean dose of 39 μg OD (range 25–50 μg) and the average final dose was 118 μg OD (range 50–225 μg).
Conclusion
Primary hypothyroidism is a common adverse effect of TKI therapy. The incidence in this cohort (20%) was similar to hypothyroidism rates in published data from Sunitinib studies (14–46%). There appeared to be a significant delay between the diagnosis of overt primary hypothyroidism and the start of LT_4. Regular pre-cycle TFT checks and close liaison with Endocrinology team will help reduce morbidity from delayed diagnosis and treatment of hypothyroidism.

DOI: 10.1530/endoabs.34.P383

P384

Mis-management of medullary thyroid carcinoma leads to investigation of a non existent phaechromocytoma

Christian Hariman, Lavanya Pelluri & Martin O'Weickert
University Hospital Coventry and Warwickshire, Coventry, UK.

Introduction
Phaeochromocytoma is a rare condition. It presents with non-specific symptoms of palpitations, headache and hypertension. Such symptoms in a patient with background of previous medullary thyroid carcinoma cannot be ignored and would warrant further investigation to exclude MEN II.
Case report
A 45-year-old gentleman presents with labile hypertension, headaches and palpitations. He has a history of a recurrent medullary thyroid carcinoma previously managed with total thyroidectomy and external beam radiotherapy. He has been on 225 μg thyroxine with a fully suppressed TSH of <0.02 mU/l. Phaeochromocytoma was suspected and investigated given his persistent symptoms. Three separate collections of urinary catecholamines have shown borderline excess production of urinary free noradrenaline between 635 and 944 nmol/collection (NR <601 nmol/collection). Subsequent imaging with an Octreotide whole body scan has shown no evidence of adrenal pathology with evidence of uptake around the supraclavicular region in keeping with a recurrence of medullary thyroid carcinoma. The patient's plasma normetanephrine was subsequently found to be normal at 585 pmol/l (NR 120–1180). This gentleman's thyroxine replacement was reduced to 175 μg to attain a biochemical euthyroid state. Both his symptoms and urinary catecholamines returned to normal as they were iatrogenic rather than due to phaeochromocytoma. Genetic testing confirmed RET oncogene as negative.
Conclusion
Biochemical investigations and confirmatory tests for phaeochromocytoma should be evaluated in light of other co-existing endocrine pathologies such as thyroxine replacement therapy. Management of medullary thyroid carcinoma with thyroxine should aim at attaining a euthyroid biochemical profile rather than supra-physiological replacement.

DOI: 10.1530/endoabs.34.P384

P385

Investigating and treating thyrotoxicosis

Momin Shah & Simon Saunders
Warrington and Halton Hospitals NHS Foundation Trust, Warrington, UK.

Aim
To evaluate the management of patients with hyperthyroidism against recommendations of the American Thyroid Association (ATA).
Methods
The case records of all new patients referred with hyperthyroidism, 02.05.2009–01.04.2011, were analysed ($n=33$), against recommendations of ATA.
Results
73% of the subjects were women. 24% patients had Graves' disease, 18% MNG, 18% thyroiditis, 15% 'autoimmune hyperthyroidism', 15% patients were broadly labelled as 'hyperthyroid' (not otherwise specified), 3% patients had amiodarone induced thyrotoxicosis, 3% thyroid hormone resistance and 3% had subclinical hyperthyroidism. Carbimazole was used in all patients with Graves' on anti-thyroid medication (100%). In patients treated with carbimazole, a baseline FBC was checked in 77% and LFTS in 51%. Smoking history was documented in 64% of patients. Only 18% had documentary evidence of advice on smoking cessation. 0% of patients with Grave' or autoimmune thyrotoxicosis had TRAB levels re-checked prior to stopping carbimazole. 33% of patients with MNG were referred for surgery (compressive symptoms) and the remainder 50% were referred for radio-iodine treatment. In patients with clinical presentation not diagnostic of Graves' disease only 16% had thyroid uptake scans. All patients with Graves' disease who were referred for radio-iodine treatment were pre-treated with carbimazole (100%).
Discussion
Anti-thyroid medication may rarely cause agranulocytosis and liver dysfunction therefore baseline FBC and LFTs should be checked in all patients. Graves' opthamopathy is six times more common in smokers; risks of smoking should be discussed and documented. Patients with persistently raised TRAB levels should be counselled regarding the high chance of relapse and will benefit from more frequent monitoring of TFTs once off anti-thyroid medication. RAI uptake should be performed as part of evaluation when the clinical presentation of thyrotoxicosis is not diagnostic of Graves' disease. Finally 'Hyperthyroidism' is not a diagnosis; every effort must be made to clarify the cause of hyperthyroidism.

DOI: 10.1530/endoabs.34.P385

P386

Prevalence of thyroid dysfunction in patients with rheumatoid arthritis

Loai Shakerdi, Wadah Haj Naema & Saied Hamdon
University of Aleppo, Aleppo, Syria.

Autoimmune diseases (ADs) are conditions under which an individual develops antibodies against their own cells, tissues and/or organ systems. Rheumatoid arthritis (RA) is a chronic multi system disease of unknown cause with multiple systemic manifestations. Autoimmune thyroid disease (ATD) are characterized by the presence of antibodies against thyroglobulin, thyroid peroxidase, or thyrotropin receptor autoantigens. The relationship between RA and the thyroid gland has been studied extensively, with several studies demonstrating the autoimmune nature of thyroid dysfunctions in RA. In the current study we ought to investigate the coexistence of thyroid dysfunction in a group of RA patients. This study was conducted at Aleppo University Hospital on RA patients attending the rheumatology outpatient clinic. Laboratory evaluation of serum FT_3, FT_4,

TSH, antimicrosomal antibodies, and antithyroglobulin antibodies levels were measured by Immunochemical analyzer Cobas e 411 HITACHI Hoffman Le Roche company (Switzerland).
The total number of RA patients was 112.7% of RA patients have thyroid dysfunction. 12.5% have hyperthyroidism, 25% have subclinical hypothyroidism, 50% hypothyroidism, and 12.5 were euthyroids. Thyroglobulin antibodies were positive in 50% of patients. Microsomal antibodies were positive in 87.5%. Rheumatic factor was positive in 87.5% of patients with thyroid dysfunction.
Our results accords with other studies in regard to the prevalence of thyroid dysfunction in RA patients. In the present study, subclinical hypothyroidism and primary hypothyroidism were the most common alterations among RA patients. These results indicate that the presence of one AD in a patient should alert the clinician to the possibility of additional ADs; and it is clinically important to screen patients with RA for the coexistence of thyroid autoimmune disease.

DOI: 10.1530/endoabs.34.P386

P387

Is there a need to change how we approach a patient with hyperthyroidism?

Lakshminarayanan Varadhan[1], Anney Altaf[2], George Varughese[1] & Sailesh Sankar[2]
[1]University Hospitals of North Staffordshire NHS Trust, Stoke on Trent, UK; [2]University Hospital Coventry and Warwickshire NHS Trust, Coventry, UK.

Aim
Establishing the aetiological diagnosis of hyperthyroidism relies on clinical examination, ultrasound imaging and thyroid antibodies; however there is a wide variation in practice in which of the aforementioned approaches are used. The aim of our study was to assess the reliability of clinical examination of thyroid gland in patients with hyperthyroidism, using ultrasound (US) as the reference.
Methods
Case notes and letters of patients of 133 patients with hyperthyroidism referred to endocrine clinic were reviewed. Patients who did not have US were excluded. Data on thyroid anatomy based on clinical examination was collected and compared to US features and clinical diagnosis of the cause of hyperthyroidism.
Results
Size: 82 patients had thyromegaly on US: only 33% of these were documented as having goitre on clinical examination. 51 patients had normal US, 35% of these were documented to have goitre on clinical examination. *Nodularity*: 54 patients had MNG on US: 30% were reported as normal and further 37% ($n=20$) were reported as having diffuse enlargement on clinical examination. five patients had solitary nodule on US; four of them were clinically documented as normal. 39 patients had normal US; nine of these patients were reported as thyromegaly and one as solitary nodule on clinical examination. *Function*: 73 patients had Graves' disease based on TPO antibody. Based on USA – normal 27%, MNG 33%, solitary nodule 5%, dominant nodule(s) of MNG 5%.
Conclusion
There is significant difference between clinical and US assessment of thyroid gland, with only 47% correlating with each other. 44% of patients with hyperthyroidism could be misdiagnosed as Graves' disease unless US and/or thyroid antibodies are performed to correlate. A combined diagnostic approach including clinical examination, US and thyroid antibodies may need to be considered in patients with hyperthyroidism to establish aetiology and formulate a management plan.

DOI: 10.1530/endoabs.34.P387

P388

A rare cause of clinical hypothyroidism: thyroid hormone resistance

Ram Prakash Narayanan[1], Preeti Chiran[1], Mohammad Al-Jubouri[2], Steven McNulty[1] & Niall Furlong[1]
[1]Department of Endocrinology, St Helens and Knowsley NHS Trust, St Helens, Merseyside, UK; [2]Department of Pathology, St Helens and Knowsley NHS Trust, Whiston, Merseyside, UK.

Thyroid hormone resistance is a rare but recognised cause of clinical hypothyroidism. We explain a case of clinical hypothyroidism that was associated with thyroid hormone resistance.

A 48-year Caucasian female with no known personal or family endocrine history was referred with a 12-month-history of weight gain and increased tiredness. Past history included depression and osteoarthritis, with regular medications being NSAIDs and amitryptiline. There was no history of investigations with iodinated contrast, recent viral illnesses or amiodarone. Initial thyroid function tests-TSH 5.3 (0.2–6), T_4 31.1(10–20) pmol/l. There was no evidence of heterophile antibody interference after polyethylene glycol precipitation tests. Similarly, repeating thyroid function tests after equilibrium dialysis (DELFIA TFT) did not alter the readings. Serum sex hormone binding globulin concentration was normal 47 (normal range 20–100) nmol/l suggesting TSHoma unlikely. Sample sent for thyroid hormone receptor gene mutation screening – heterozygous for *THRB* C.1313G>A single base change mutation (Arg 438 His). Patient referred to the local clinical genetics service for family screening. Commenced on 25 μg of levothyroxine with a view to alleviate symptoms.
Conclusion
Clinical hypothyroidism with elevated TFTs should suggest the possibility of thyroid hormone resistance and be investigated accordingly.

DOI: 10.1530/endoabs.34.P388

P389

Radioiodine therapy for thyrotoxicosis over a 2-year period: an audit

Jennifer Clark[2], Ranganatha Rao[1], Prabir Bandyopadhyay[1] & Parijat De[1]
[1]Department of Diabetes & Endocrinology, Sandwell & West Birmingham Hospital Trust, Birmingham, UK; [2]University of Birmingham, Birmingham, UK.

Aims
To look at the demographics, biochemistry, treatments used, clinical characteristics associated with radioiodine (RI) treatment (including those with relapse) for thyrotoxicosis in our Endocrine clinic.
Methods
Electronic records of all thyrotoxic patients between 2010 and 2012.
Results
144 patients, mean age 56 years (114 females)–106 Graves, 24 toxic adenoma (TA) and 14 multi nodular goitre (MNG). TPO antibody was positive in 104/144 (72%) patients. At baseline, 6 and 12 months respectively mean FT_4 was 23.4, 15.25 7 16.9 pmol, mean FT_3 was 8.3, 5.9 and 5.6 pmol and TSH 1.09, 25.5 and 8.13 IU/l.
26/144(18%) had dysthyroid eye disease and 15/144 (10%) post radioiodine treatment (only three patients needed steroids). 7/26 (3%) with eye disease were smokers. 24/31(77%) smokers had no eye problems. Mean anti thyroid drug (ATD) duration was 12.7 months before RI (25/144 – 17% did not need RI).
Transient hypothyroidism developed in 57/119 (48%) patients, 5 months after RI. Permanent hypothyroidism developed in 62/119 (52%) patients, average 5 month after RI treatment.
6/119 (5%) needed second RI dose – average age 57 years, all females, mean FT_4 at baseline was higher (44 vs 23.4 pmol whole group), mean ATD duration was higher (42 vs 12.7 months whole group), three had TA and three Graves, all six were pre treated with carbimazole (two also needed propylthiouracil) and the average gap between two RI treatments was 2 years.
Conclusions
83% of our thyrotoxic patients needed RI following 12 months of ATD treatment. Prevalence of transient hypothyroidism was expected but 50% developed permanent hypothyroidism within 6 months of RI use – likely related to the higher radioablative dose currently being used (600–650 MBq). Perhaps for the same reason, only 5% of our cohort required second RI dose – these patients had a much higher baseline FT_4 and needed ATD for at least 3.5 years.

DOI: 10.1530/endoabs.34.P389

P390

Factors contributing to high levothyroxine doses in primary hypothyroidism; an interventional audit of a large community database

Hannah Robertson[1], Anil Narayanaswamy[2], Olivia Pereira[3], Shirley Copland[1], Richard Herriot[1], Alastair McKinlay[1], John Bevan[1] & Prakash Abraham[1]
[1]Aberdeen Royal Infirmary, Aberdeen, UK; [2]Centre for Endocrine and Diabetes Sciences, University Hospital of Wales, Cardiff, UK; [3]Edna Coates Diabetes Centre, Pinderfields Hospital, Wakefield, UK.

Background
While few hypothyroid patients require more than the expected weight related dose of levothyroxine, the underlying causes of larger-than-expected dosing requirements have not been studied in a single cohort. Our aim was to determine and quantify the multiple factors contributing to high dose levothyroxine requirements in a cohort of patients with hypothyroidism.
Methods
The Grampian Automated Follow-Up Register (GAFUR) monitors around 17 500 hypothyroid patients; in 2008, 190 (1%) patients took more than 225 μg of levothyroxine daily. A questionnaire was sent to 174 patients (16 were untraceable) to assess causes and to offer blood tests for endomysial, parietal cell (PCA) and thyroid peroxidase (TPO) auto-antibodies. Primary care surgeries were contacted for medication details. All patients with positive endomysial autoantibodies were referred to a gastroenterologist. Thyroid function tests and levothyroxine doses were re-evaluated in 2011.
Results
125 questionnaires (72%) were returned. Mean levothyroxine dose was 248 μg daily. 26 patients (20.8%) took medications known to interfere with levothyroxine absorption and 21 patients (16.8%) admitted to compliance issues. Seven patients had positive anti-endomysial antibodies on initial screening with four being new diagnoses of celiac disease and PCA were positive in 27 (21.6%) patients. At follow-up in 2011 the mean levothyroxine dose had decreased in patients on interfering medications and in the four new cases of coeliac disease.
Conclusions
Causes of patients needing high dose levothyroxine replacement include poor compliance, medication interference, PCA (as a marker of atrophic/autoimmune gastritis) and coeliac disease. Doses can be decreased with counselling regarding medications or after management of underlying conditions.

DOI: 10.1530/endoabs.34.P390

P391

Anti-thyroid drugs as treatment for neutropenia in Graves' thyrotoxicosis

N Aggarwal[1], W Saqib[1], T Fretwell[1], G Summerfield[1] & S Razvi[1,2]
[1]Queen Elizabeth Hospital, Gateshead, UK; [2]Institute of Human Genetics, Newcastle University, Newcastle upon Tyne, UK.

Background
Neutropenia due to anti-thyroid drug (ATD) therapy in Graves' disease is rare but is well recognised. However, the effect of hyperthyroidism, prior to and after ATD therapy, on absolute neutrophil counts in patients with Graves' disease is unclear.
Methods
We noted neutrophil levels in consecutive patients with newly diagnosed Graves' thyrotoxicosis in 2010–2013. We further noted neutrophil levels once patients had been treated with ATD for at least 3 months and were rendered euthyroid. A neutrophil count of $<2\times10^9$/l was classed as neutropenia. Neutrophil levels at baseline and after euthyroidism was achieved were compared by paired Student's *t*-test. Multivariable linear regression analysis was performed to ascertain factors affecting change in neutrophil levels.
Results
At initial diagnosis of Graves' thyrotoxicosis and prior to initiation of ATD, 24/147 (16.3%) individuals had neutropaenia. Compared to individuals without, those with neutropenia were more likely to be younger (42.1 vs 50.3 years; $P=0.02$), have more severe hyperthyroidism (FT_4 levels of 67.6 vs 45.3 pmol/l; $P<0.001$), less likely to be current smokers (20.8 vs 34.1%; $P<0.01$) and have Graves' orbitopathy (25 vs 15.4%; $P=0.01$). After multivariable regression analysis, severity of hyperthyroidism ($P<0.005$) and non smoking status ($P=0.02$) were the only independent predictors of neutropenia and there was no association with TBII levels. After euthyroidism was achieved, none of the patients in the neutropaenic group ($n=16$) remained neutropaenic. Furthermore, neutrophil levels increased significantly (3.8 vs 4.8 10^9/l; $P<0.001$) even in patients that were not neutropenic at baseline ($n=74$). Improvement in neutrophil count was related to reduction in thyroid hormone levels ($P<0.001$) and was more pronounced in males.
Conclusions
Graves' thyrotoxicosis is associated with neutropenia in about a sixth of patients at diagnosis. This improves with treatment with ATD due to reduction in thyroid hormone concentrations. It is therefore crucial to check neutrophil levels in newly diagnosed patients with Graves' thyrotoxicosis prior to commencing ATD therapy as otherwise low levels may incorrectly be attributed to ATD therapy.

DOI: 10.1530/endoabs.34.P391

P392

A case of newly diagnosed hyperthyroidism in the 25th gestational week of pregnancy presented with divergent arterial hypertension

Mateja Legan[1], Janez Zaveljcina[2] & Simona Gaberscek[2]
[1]Division of Gynaecology, University Medical Centre Ljubljana, Ljubljana, Slovenia; [2]Department of Nuclear Medicine, University Medical Centre Ljubljana, Ljubljana, Slovenia.

Background
During pregnancy, the immune system is suppressed. Therefore, autoimmune thyroid disorders (AITD) rarely appear in that period for the first time.
Case report
We present a case of a 30-year-old female firstly referred to our department in the 25th week of pregnancy because of 14-day-lasting arterial hypertension. At the systolic blood pressure between 140 and 160 mmHg, her dyastolic blood pressure was between 60 and 70 mmHg. She had been normotensive before hand and during previous pregnancy. She had no other symptoms or signs of hyperthyroidism. Laboratory tests revealed hyperthyroidism: TSH 0.005 mU/l (normal range 0.35–5.5 mU/l), free T_4 28.6 pmol/l (normal range 11.5–22.7 pmol/l), and free T_3 11.5 pmol/l (normal range 3.5–6.5 pmol/l). Thyroid gland was ultrasonographically enlarged, hypoechoic and lively perfused. Thyroid peroxidase antibodies were above 1300 KU/l (normal value below 60 KU/l), and TSH receptor antibodies 1 U/l (normal value below 1.5 U/l) Treatment with 3×50 mg of propilthiouracil daily was started. For arterial hypertension, methyldopa was introduced. One month later, free thyroid hormones and blood pressure normalized. In the 40th gestational week, she delivered a healthy baby daughter. Three weeks after delivery, she was normotensive without antihypertensive drugs. propilthiouracil was discontinued. In the next 4 months, she developed hypothyroidism and a substitution with L-thyroxine was started. The course of the disease revealed Hashimoto's thyroiditis (HT) as a cause of hyperthyroidism in pregnancy, followed by a hypothyroidism after delivery.
Conclusion
Two important messages can be drawn from our case report. First, a divergent arterial hypertension in pregnancy can be the only and the warning sign of hyperthyroidism in that period. Secondly, in the second half of pregnancy, a hyperthyroid phase of HT can occur in spite of well-known amelioration of AITD in that period.

DOI: 10.1530/endoabs.34.P392

P393

Deafness and goitre: think pendred

Jasvir Virdee, Russell Warwick, Ranganatha Rao, Robert Ryder & Parijat De
City Hospital, Birmingham, UK.

Introduction
Pendred syndrome is a rare autosomal recessive condition characterised by sensorineural deafness, goitre and impaired iodine organification. We report a case of a 43-year-old female who presented with hearing impairment, and the development of a multinodular goitre with tracheal compression. Perchlorate testing and genetic studies confirmed a diagnosis of Pendred syndrome. She subsequently underwent partial thyroidectomy and requires lifelong follow-up.
Case report
A 43-year-old Afro-Caribbean female presented at age 30 in our endocrine clinic with a 15-year history of goitre, where she described only hoarseness of voice.

She has a background of childhood deafness bilaterally, a presumed complication of measles. She was noted to have negative thyroid autoantibodies, TSH 2.7 mU/l and FT_4 10 pmol/l. She was discharged, but re-referred 8 years later due to increasing goitre size, resulting in dysphagia and difficulties breathing. Ultrasound and CT imaging revealed a sizeable multinodular goitre with right-sided tracheal deviation and lateral compression. She underwent a left lobectomy and isthmusectomy, but suffered vocal cord palsy complications, and did not undergo a right lobectomy. A positive perchlorate test followed by genetic studies, which demonstrated homozygous c.1151A>G, confirmed the diagnosis of Pendred syndrome. She requires lifelong follow-up.

Discussion

The combination of sensorineural deafness and goitre should alert the clinician to the possibility of Pendred syndrome, which is due to biallelic mutations in the SLCC26A4 gene. As the condition is autosomal recessive, a full family history should be taken. Although the thyroid goitre is usually benign, hypothyroidism occurs in up to 40% of cases. Even following surgery, re-growth of goitre can occur. The perchlorate test, a radiolabelled iodine scan, aids in the diagnosis of Pendred syndrome, which shows poor orgnanification of iodine. Genetic testing (homozygosity of c.1151A>G) confirms the diagnosis.

DOI: 10.1530/endoabs.34.P393

P394

PBF is a component of the molecular signalling pathways that drive hyperplastic and neoplastic thyroid growth

Vicki Smith, Martin Read, Rachel Watkins, Vikki Poole, Bhavika Modasia, Waraporn Imruetaicharoenchoke, Kristien Boelaert, Jayne Franklyn & Christopher McCabe
University of Birmingham, Birmingham, UK.

Thyroid growth and differentiation are regulated by TSH via its receptor (TSHR), whilst growth factors signal in parallel via the MAPK/ERK and PI3K/AKT pathways. Aberrant thyroid growth is largely driven by molecular alterations within these signalling pathways. The proto-oncogene pituitary tumor-transforming gene-binding factor (PBF) is expressed in normal thyroid and upregulated in human goitre and thyroid cancer. High PBF expression is associated with tumour recurrence, distant metastasis and reduced survival in thyroid cancer. In experimental models, PBF induces goitre and tumours, and is pro-invasive. We recently demonstrated that phosphorylation of tyrosine residue 174 is mediated by the proto-oncogene protein tyrosine kinase Src. This prompted further investigation into a role for PBF in thyroid signalling pathways. PBF was phosphorylated *in vivo* in our mouse model of thyroid-specific PBF over-expression (PBF-Tg), accompanied by increased TSHR and cyclin D1 expression, and pAkt induction. *In vitro* studies showed that MAPK pathway stimulation with the BRAF V600E mutant, the most prevalent thyroid cancer mutation, results in increased PBF expression and phosphorylation, signifying a role for PBF in this critical thyroid signalling pathway. Epithelial–mesenchymal transition (EMT) is a process by which cells lose cell–cell contact, becoming migratory and invasive, and is governed by TGF-β, Runx2, Src and MAPK signalling in thyroid cancer. TGF-β treatment induced PBF phosphorylation, and Runx2 overexpression upregulated PBF expression *in vitro*. Importantly, reduced E-cadherin expression, a hallmark of EMT, was evident in the PBF-Tg mouse thyroid. We propose therefore that PBF is a component of the central thyroid signalling pathways that mediate normal thyroid growth and function, and that dysregulation of these pathways in nodular goitre and thyroid cancer induces PBF expression and phosphorylation. Further, we hypothesise that PBF may promote EMT-driven thyroid cancer progression, and is consequently both a negative prognostic factor and a putative therapeutic target for aggressive thyroid tumours.

DOI: 10.1530/endoabs.34.P394

P395

Clinical outcome of radioiodine treatment for Graves' disease at a tertiary care centre

Ali Rathore, Rammya Mathew, Anukul Garg & Mark Vanderpump
Royal Free Hospital, London, UK.

Objective

A review of the efficacy and safety of radioiodine treatment for Graves' disease.

Methodology

Patients were identified following a search of the nuclear medicine departmental database. Consecutive patients who had radioiodine in 3-year period from January 2009 to December 2011 were included in the audit. Information was obtained from medical notes, blood results and nuclear medicine database. Data was analysed using Microsoft Excel.

Results

50 patients (34% men; and 66% women) had radioiodine for Graves' disease over 3-year period. The mean age was 47 years (27–90). Four patients (8%) had previously received a dose of radioiodine. 91% had received anti-thyroid drugs using a titration regime. None had a previous thyroidectomy. After radioiodine, 30% required further treatment with anti-thyroid drugs. At 2-year follow-up, 86% had developed hypothyroidism, one patient remained euthyroid and 12% remained hyperthyroid. Among the four patients who were receiving a second dose of radioiodine, three became hypothyroid and one remained hyperthyroid. The average time of diagnosis of hypothyroidism was 5 months post radioiodine (2–11.5 months). At 12-month follow-up, 69% of patients had gained weight and 31% had lost weight. Overall there was an average weight gain of 2.65 kg. Men had slightly more average weight gain than women (3.72 vs 2.17 kg). Patients over 50 years of age had a greater average weight gain compared to those aged 50 or less (4.01 vs 1.06 kg). Eleven patients had pre-existing orbitopathy of which seven patients received steroid prophylaxis. No exacerbation of orbitopathy was reported. One case of new orbitopathy was diagnosed on follow-up where only lubricating eye drops were required. Thyroiditis was diagnosed in three patients. No other complications were noted.

Conclusions

Radioiodine treatment for Graves' disease has been proven to be an effective and safe treatment modality. The overall success rate in achieving hypothyroidism or euthyroidism was 88%. Weight gain was variable. In general, male gender and older age were associated with greater weight gain. There was no exacerbation of pre-existing orbitopathy despite variations in practice regarding steroid prophylaxis.

DOI: 10.1530/endoabs.34.P395

P396

A highly exaggerated response to Warfarin therapy in a patient with thyrotoxicosis

Tolulope Shonibare, Elizabeth Berkin, Bernard Chang & Ramzi Ajjan
Department of Endocrinology and Diabetes, St James University Hospital, Leeds, UK.

Case history

A 22-year-old lady was referred to the thyroid eye clinic with bilateral proptosis following a routine visit to the opticians. She had a 2-year history of thyrotoxic symptoms, which had been getting worse over the past 4 months.

She had signs of severe thyrotoxicosis and was clinically in atrial fibrillation (AF) with a central heart rate of 195 beats/min. Neck palpation revealed a moderate-sized smooth and symmetrical goitre with no bruits or lymphadenopathy. There was evidence of active thyroid eye disease with a clinical disease activity score of 3. Her investigations revealed a free T_4 109.8 pmol/l, total T_3>12.3 nmol/l and TSH < 0.05 mIU/l. Her TSH-R antibodies were positive at 100 U/l, consistent with a diagnosis of Graves' disease, and her electrocardiogram (ECG) confirmed AF. She was commenced on carbimazole 20 mg three times daily and 40 mg/day of prednisolone was added for the management of thyroid eye disease. Following advice from cardiology, she was started on 5 mg bisoprolol and warfarin was added using the standard Fennerty protocol. Her INR after three doses of warfarin was 18.7 but the patient remained asymptomatic. Warfarin was stopped and the dose was subsequently adjusted to achieve an INR between 2 and 3. She became euthyroid in 6 weeks and reverted spontaneously into stable sinus rhythm, confirmed by 24 h ECG.

Discussion

A limited number of reports have shown an increased sensitivity to oral anticoagulants in thyrotoxicosis, but to our knowledge, this case represents the most extreme case described to date. The mechanism remains unclear, although some have suggested thyroxine induced alteration in clotting factor and/or altered metabolism of warfarin. In our patient, the INR was nearly tenfold above the therapeutic range, despite being induced on the standard Fennerty warfarin protocol. This highlights the need for a different anticoagulation regimen in patients with thyrotoxicosis and AF. We suggest more frequent and earlier checks of INR in cases where warfarin therapy is required in patients with hyperthyroidism, particularly in those with severe thyrotoxicosis.

DOI: 10.1530/endoabs.34.P396

P397

Examination of PTTG-binding factor mRNA reveals functional miRNA target sites and exon-skipping splice variants in thyroid cells

Martin Read, Jo Longman, Alice Fletcher, Vicki Smith, Rachel Watkins, Bhavika Modasia, Vikki Poole, Waraporn Imruetaicharoenchoke, Jayne Franklyn, Kristien Boelaert & Christopher McCabe
University of Birmingham, Birmingham, UK.

Dysregulation of the processing and stability of mRNA encoding oncogenes or tumour suppressor proteins is a critical event in the pathogenesis of cancer. Previously, we demonstrated that the proto-oncogene PTTG-binding factor (PBF) is overexpressed in thyroid, pituitary and breast tumours. Critically, high PBF expression is associated with reduced disease-specific survival in thyroid cancer. However, the mechanisms responsible for regulating PBF expression are unknown. In this study, we examined PBF mRNA in human thyroid cancer FFPE specimens by touchdown 1-step RT-PCR analysis. Four different PBF mRNA splice variants (SV) were identified through direct sequencing. Altered expression of an SV lacking exons 4 and 5 was evident in 27/30 thyroid tumours, in two main groups with either up- (1.5 ± 0.2; $n=9$) or down-regulation (0.42 ± 0.06; $n=18$), and both with a significant twofold difference in relative expression compared to full-length PBF mRNA ($P<0.05$). We next investigated whether PBF mRNA contained any target sites for regulatory miRNAs that might induce mRNA degradation. Bioinformatic analysis revealed a number of putative miRNA interaction sites in the 3' UTR region of the PBF gene. Five miRNAs (i.e. miR-122, -124, -365, -506 and -647) were selected for further study based on evolutionary conservation of sequence. Importantly, a significant reduction in endogenous PBF mRNA levels was observed following transfection of thyroid cancer TPC-1 cells using synthetic mimics for miR-506-3p ($56.9 \pm 4.0\%$; $P<0.001$), miR-122-5p ($52.5 \pm 10.6\%$; $P<0.01$) and miR-124-3p ($40.6 \pm 5.6\%$; $P<0.01$) after 48 hr. In contrast, there was no change with miR-647 ($P=$NS) or miR-365a-3p ($P=$NS). In summary, these results implicate exon-skipping as a possible mechanism in dysregulating PBF activity in thyroid cells. Furthermore, our findings suggest that the stability of PBF mRNA is dependent on miRNAs such as miR-506 and miR-124, the expression of which are frequently down-regulated in tumours and associated with increased cell motility and invasion capability of malignant cells.

DOI: 10.1530/endoabs.34.P397

P398

Neurocognitive and CNS abnormalities in humans with defective thyroid receptor α

Carla Moran[1], Edward Visser[1], Nadia Schoenmakers[1], Francesco Muntoni[2], Chris Clark[3], David Gadian[3], Kling Chong[4], Adam Kuczynski[5], Mehul Dattani[6], Faraneh Vargha-Khadem[5] & Krishna Chatterjee[1]
[1]Wellcome-MRC Insitute of Metabolic Science, University of Cambridge, Cambridge, UK; [2]Dubowitz Neuromuscular Centre, UCL Institute of Child Health, London, UK; [3]Imaging and Biophysics, UCL Institute of Child Health, London, UK; [4]Department of Radiology, Great Ormond St Children's Hospital, London, UK; [5]Department of Neuropsychology, Great Ormond St Children's Hospital, London, UK; [6]Department of Endocrinlogy, Great Ormond St Children's Hospital, London, UK.

Background
The severe neurodevelopmental phenotype of untreated congenital hypothyroidism exemplifies the critical role of thyroid hormones (TH) in CNS development, acting via thyroid hormone receptor α1 (TRα1) on cortical neurogenesis, cerebellar development and oligodendrocyte differentiation. We have identified the first humans with defective TRα1 and investigated neurocognitive phenotype and CNS abnormalities in this disorder.
Methods
Four affected individuals (P1: female 8 years; P2: female 13 years; P3: father of P2 53 years; P4: female 43 years) were studied. Neurological investigation, cognitive testing, and brain magnetic resonance imaging and spectroscopy (MRI/S) were undertaken.
Findings
All subjects exhibited developmental delay; P4 also has severe learning disability and epilepsy. Neurological abnormalities common to all cases include gross and fine motor incoordination, with ataxic gait, dysdiadochokinesis and slow speech, associated with reduced cerebellar volume on MRI. IQ is variably reduced (P1 84, P2 90, P3 85, P4 52) and adult cases exhibit marked microencephaly; reduced *N*-acetylaspartate (NAA) levels (expressed as NAA:creatine ratio) measured by magnetic resonance spectroscopy (frontal white matter: P3 1.77, P4 1.54, controls $2.2 +/- 0.2$); thalamic: P3 2.05, P4 1.9, controls 2.09–2.25) suggests neuronal loss or dysfunction. Diffusion tensor imaging (P1, P2) indicates reduced axonal density/myelination and tract organisation; impaired verbal longterm memory (P1) correlates with significant reduction (20%) in hippocampal volume. Known neural target genes (hairless, KLF-9) in patient derived mononuclear cells are TH refractory.
Interpretation
Observed neurocognitive deficits (motor incoordination, reduced IQ, impaired longterm memory) and structural abnormalities (microcephaly, reduced cerebellar and hippocampal volume, diminished white matter density) accord with known developmental actions of thyroid hormone and substantiate the critical CNS role of TRα1. Studies of neural cell types generated from their inducible pluripotent stem cells may elucidate TH-dependent brain pathways.

DOI: 10.1530/endoabs.34.P398

P399

Thyrotoxicosis: a district general hospital experience compared to guidelines

Emily Brown, Momin Shah, Pallavi Hegde & Simon Saunders
Warrington Hospital, Cheshire, UK.

Aims
To assess the diagnosis and management of patients with thyrotoxicosis in a district general hospital comparing with American Thyroid Association (ATA) recommendations.
Methods
Retrospective study, electronic case notes of patients referred to the endocrine clinic between May 2009 and April 2011 ($n=148$) were reviewed. A pro-forma was designed collecting details of demographics, investigations, diagnosis and treatments received.
Results
33 patients with thyrotoxicosis identified; 24 (77%) female, 9 (23%) male. 8 (24%) diagnosed with Graves' disease (GD), 6 (18%) autoimmune hyperthyroidism, 6 (18%) toxic multinodular goitre (TMNG), 5 (15%) unspecified and 3 (9%) other. The average number of appointments made prior to discharge was 5.3. All but one patient with clinical features of GD had confirmatory thyrotropin receptor antibodies (TRAb) testing. 4/25 (16%) patients without features of GD were referred for a radioactive iodine uptake scan (RAIU).
Anti-thyroid medications were initiated in 27 (82%) patients. All 27 were commenced on titrated doses of carbimazole (CBZ) of which 21 (77%) and 14 (49%) had baseline FBC and LFTs checked respectively. Definitive treatment with radioiodine was given to one patient diagnosed with relapsed GD after pre-treatment with CBZ. 3/6 (83%) patients diagnosed with TMNG were directly referred for either radioiodine therapy or surgery and started on pre-treatment with CBZ. Two relapsed after reducing regimes of CBZ and were consequently referred for radioiodine therapy. One patient chose to stay on low dose CBZ. 0/8 (0%) patients diagnosed with GD had TRAb testing prior to discontinuation of treatment.
Conclusion
Attempts to make an accurate diagnosis should be made. Ultimately 'patient choice' guides management but use of definitive treatment such as radio-iodine/surgery should be considered. Measurement of TRAb levels prior to stopping oral treatment is recommended to aid the decision to stop treatment and chance of remission. An automated computerized thyroid register would aid monitoring and reduce the number of outpatient appointments.

DOI: 10.1530/endoabs.34.P399

P400

A case of Hashimoto encephalitis in a patient with severe autoimmune hypothyroidism

Jana Bujanova, Shiva Mongolu & Azraai Nasruddin
Endocrinology Department, Southampton General Hospital, Southampton, UK.

We present a case of 76-year-old male who attended acute medical unit with 3 week history of confusion, slurred speech and reduced mobility. One month prior to his admission he was diagnosed with severe hypothyroidism with TSH of 100 mU/l and T_4 of 1.9 pmol/l and significantly positive anti-peroxidase antibodies (561 IU/ml) and was started on 100 μg of levothyroxine by his GP. During his admission his confusion persisted despite antibiotics for presumed chest infection. He had associated hyponatraemia and postural blood pressure drop of 40 mmHg. His Short Synacthen test was abnormal with baseline and

stimulated cortisol of 55 and 130 nmol/l respectively. Diagnosis of Addison's disease was made and this was most likely exacerbated by infection and recently commenced levothyroxine. He was also found to have primary testicular failure (testosterone, 4.6 nmol/l; FSH, 63.6 IU/l; LH, 32.3 IU/l) with anaemia. Lumbar puncture was performed and showed raised CSF protein of 1917 mg/l. Bacterial and viral CNS infection was excluded. EEG was in keeping with mild encephalopathy and MRI showed periventricular white matter changes associated with inflammation. Diagnosis of Hashimoto encephalitis was made after exclusion of other causes of encephalitis. Patient was started on prednisolone 40 mg od and his confusion fully resolved after 2 months of therapy. Dose was down-titrated to 5 mg od over 12 months.

Discussion

Hashimoto encephalitis is a diagnosis of exclusion in confused patients with autoimmune hypothyroidism and should be kept in mind as the prognosis is good with prompt steroid therapy. In this case, it was a part of a complex autoimmune syndrome. Presenting symptoms include confusion, seizures, psychosis and stroke like symptoms. There is no evidence of the pathogenic role of the thyroid antibodies and these are probably markers of some other autoimmune process affecting the brain.

DOI: 10.1530/endoabs.34.P400

P401

The diagnosis of Graves' disease: diagnostic and management dilemmas

Lakshminarayanan Pichaipillai[1,2] & Biswa Mishra[1]
[1]The Penine Acute Hospitals NHS Trust, Oldham, Lancashire, UK; [2]Royal Blackburn Hospital, Blackburn, Lancashire, UK.

Aim

Diagnosis of Graves' is generally based on clinical presentation and physical examination of thyroid gland. The general assumption is that the thyroid gland is usually normal or diffusely enlarged in Graves' disease. The aim of our retrospective observational analysis was to establish the ultrasonographic morphology of the thyroid gland in patients with Graves' disease.

Methods

We analysed the case records and biochemical investigations of all patients with thyrotoxicosis presenting to the Endocrinology out-patient of a district general hospital over the last 4 years (2009–2012). The diagnosis of Graves' disease was confirmed by the presence of TSH receptor antibody (TRAB). Patients who had both positive TRAB antibody (considered to be diagnostic of Graves' disease with specificity 98% as per literature) and US of the thyroid gland were included for analysis.

Results

433 patients were diagnosed as thyrotoxic. TRAB was positive in 199 patients. Ultrasound of the thyroid was done in 126 out of 199 TRAB positive patients. 53% (N-67) patients had typical features expected of Graves' on US namely normal gland, diffuse enlargement, asymmetrical enlargement or features of thyroiditis. However, 47% (N-59) patients had demonstrated nodularity on USG: 95% (N-58) of these patients had multinodular goitre (MNG), of whom 23 patients had dominant nodule in the MNG and the rest had MNG without any dominant nodule. 5% (N-3) patients had solitary nodule.

Conclusion

This retrospective analysis demonstrates the diverse morphology of thyroid gland in patients with Graves' disease. Coexistent thyroid nodules may warrant further evaluation with Uptake scan or biopsy. Our study highlights the need to consider ultrasound as an essential part of evaluation of Graves' disease. This is even more pertinent in hospitals where TRAB is not routinely available.

DOI: 10.1530/endoabs.34.P401

P402

Severe transient hyperthyroidism and Wernicke's encephalopathy in a lady with hyperemesis gravidarum

Mehjabeen Beebeejaun, Susanna Meade, Emma Bingham, Frances Coyle & Jennifer Tringham
Frimley Park Hospital, Surrey, UK.

Transient mild hyperthyroidism is fairly common in women with hyperemesis gravidarum, occurring in up to two-thirds of them. It is also a well known fact that pregnant women with severe hyperemesis gravidarum can progress to develop Wernicke's encephalopathy. Hyperthyroidism, without any other risk factors, has been described to accelerate the process of thiamine depletion as well. This is much less recognised.

We describe the case of a 32-year-old Pakistani lady who presented with severe transient hyperthyroidism associated with hyperemesis resulting in rapidly developing neurological sequelae with typical radiological appearances of Wernicke's encephalopathy. She recovered promptly with electrolyte and thiamine replacement as well as institution of treatment with propylthiouracil.

Our case therefore reflects two metabolic stresses, hyperthyroidism and hyperemesis, precipitating Wernicke's encephalopathy.

DOI: 10.1530/endoabs.34.P402

P403

Can a radiological scoring system for assessing the malignant potential of thyroid nodules be safely applied in clinical practice?

Mark Lewis[1,2], Sunethra Ghattamaneni[1], Annice Mukherjee[1,2], Helen Doran[1,2] & Seema Dhatta[1]
[1]Salford Royal NHS Foundation Trust, Salford, Lancashire, UK; [2]The University of Manchester, Manchester, Lancashire, UK.

Background

The majority of thyroid nodules are clinically insignificant, however considerable overlap in radiological characteristics exists for benign and malignant lesions. Recently, validated radiological criteria using thyroid imaging reporting and data system scoring (TIRADS) have shown correlation between cumulative suspicious features and risk of malignancy[1].

Objective

To assess sensitivity, specificity and reliability of ultrasound scan (USS) in evaluation and differentiation of benign from malignant thyroid lesions.

Methods

Patients presenting with a thyroid nodule between 2007 and 2012 to the thyroid service at our institution and in whom surgical histology data was subsequently available were studied. Pre-operative thyroid ultrasound images were anonymised and retrospectively reviewed by two independent, blinded, radiologists. Reports were generated for individual and overall TIRADS score. Only static images were available for analysis. TIRADS scores ranged from 1 to 5. Suspicious nodules scored between 4a–c and 5. TIRADS scores of 1–3 were considered benign. Final histology was used as the definitive outcome variable. TIRADS sensitivity and specificity for malignancy was determined by dichotomising scores between 'benign' and 'malignant' and shifting the level at which nodules were considered malignant.

Results

58 patients (45F and 13M), with 71 nodules, 49 benign, and 22 malignant nodules were studied. Eighteen patients had a malignancy, of which three were multifocal. A TIRADS score of 4a or higher yielded sensitivity for malignancy of 68.2–81.8%; 5.63–9.86% of malignancies were missed by this algorithm (range represents reporting discrepancies between radiologists).

Conclusions

In our centre the TIRADS diagnostic accuracy on static ultrasound appeared inferior to current established diagnostic techniques.

Prospective use of TIRADS using dynamic images alongside current clinical diagnostic techniques requires further study.

Reference

1. Kwak JY *et al.* Thyroid imaging reporting and data system for US features of nodules: a step in establishing better stratification of cancer risk. *Radiology* 2011 **260**(3) 892–899.

DOI: 10.1530/endoabs.34.P403

P404

Thyroid nodules: Is one robust Thy2 result sufficient?

Jalini Joharatnam, Rose Ngu, Ash Chandra & Jake Powrie
Guy's and St Thomas NHS Foundation Trust, London, UK.

Introduction

Approximately 400 thyroid fine needle aspirates (FNAs) are performed annually at our institution. Many are repeat aspirates of Thy2 results. Current British Thyroid Association (BTA) and Royal College of Pathologists (RCPath) guidelines recommend two FNAs performed at least 6 months apart to confirm a benign result. Our aim was to investigate whether one Thy2 result is sufficient to confirm benign pathology.

Method

A retrospective analysis was performed on all 416 thyroid FNAs carried out in the year 2010. 312 of these were Thy2. Of these Thy2 results only 92 patients were subject to a second confirmatory FNA. Nine patients had two distinct nodules that were sampled twice giving 101 repeat sampling events.

Results
The second FNA was Thy1 in nine cases, Thy2 in 86 cases, and Thy3 in six cases. Of the six Thy3 results, all had surgery and histology revealed five were benign and one malignant. In the malignant case, the first ultrasound guided FNA of the nodule although reported as Thy2 was said to show granulomatous thyroiditis. After an interval review the lump was noted to be hard and fixed. Ultrasound showed increase in size with irregular margins and repeat FNA showed lymphoid cells raising the possibility of lymphoma. Thyroidectomy revealed diffuse large B-cell lymphoma.
Conclusion
We perform repeat FNAs in only 32% of initial Thy2 results with a very low false negative rate. We propose that, in the majority of thyroid nodules, one Thy2 ultrasound guided thyroid FNA result is sufficient to make a robust benign diagnosis. Repeat testing should only be performed in those patients with suspicious or higher risk clinical or US features.

DOI: 10.1530/endoabs.34.P404

P405

Scalp metastasis in follicular thyroid cancer: an atypical consequence

Anne Marie Hannon, Frank O'Brien, Deirdre Fitzgerald & Antoinette Tuthill
Cork University Hospital, Cork, Ireland.

Follicular thyroid cancer is the second most common cause of thyroid carcinoma. Metastasis occurs in 10–15% of cases. Typical sites of metastasis include bone and lungs, scalp metastasis are rare, with <50 cases reported. We describe two such cases.
A retrospective chart review was performed on these patients, examining presentation, treatment and subsequent outcomes.
Patient 1. An abnormal lesion was noted on a routine chest X-ray, this was biopsied via bronchoscopy. Histology revealed follicular thyroid carcinoma and she proceeded to total thyroidectomy. This confirmed follicular thyroid cell carcinoma. Whole body iodine scanning showed activity in the salivary glands and lungs. She underwent iodine ablation therapy, but in the following months she noticed the development of a scalp lesion, resembling an ulcer. This was biopsied, revealing metastatic follicular thyroid cell carcinoma.
Patient 2 presented with back pain. As part of the diagnostic workup, she underwent an MRI spine, which revealed a metastatic deposit at the level of L2. This was biopsied which showed follicular thyroid tissue, consistent with metastatic disease. She underwent a total thyroidectomy. Following this, whole body iodine scanning revealed uptake in the thyroid bed, lungs and scalp. The scalp uptake was consistent with cutaneous metastasis and the scalp lesion resolved with radioactive iodine ablation therapy.
These cases describe the uncommon occurrence of scalp metastasis in patients with follicular thyroid carcinoma. Scalp metastasis are associated with poor prognosis. Of note both cases presented with advanced secondary disease, which is consistent with previous cases described in the literature.

DOI: 10.1530/endoabs.34.P405

P406

Hypoxic incubation favours the development and growth of primary human parathyroid adenoma cells

Kate E Lines[1], Mark Stevenson[1], Radu Mihai[1], Irina V Grigorieva[1], Dilair Baban[2], Katherine U Gaynor[1], Jeshmi Jeyabalan[1], Mahsa Javid[1] & Rajesh V Thakker[1]
[1]OCDEM, University of Oxford, Oxford, UK; [2]Oxford Genomics Centre, University of Oxford, Oxford, UK.

Hypoxia is a primary stimulus for angiogenesis, which is important for tumour proliferation and survival. The effects of hypoxia on parathyroid tumour cells, which may be important for parathyroid autotransplantation in patients, are however, not known. We therefore assessed the effects of hypoxia on gene expression in parathyroid adenoma cells from patients with primary hyperparathyoridism. Cell suspensions from human parathyroid adenomas ($n=5$) were cultured and after selection in low calcium containing media, some cells were incubated under normoxic (21% oxygen) and some hypoxic (1% oxygen) conditions, for 48 h. cDNA expression analysis using Human WG6_v3 Illumina expression bead chips was performed on RNA isolated from these paired normoxic and hypoxic parathyroid cells, and the results of selected genes confirmed and validated by qRT-PCR on up to eight other parathyroid adenoma cultures. In total 549 genes were up-regulated and 873 down-regulated (>1.5-fold change; $P<0.05$). Amongst the most highly up-regulated genes (greater than fivefold) were those involved in hypoxia (*CA9*, *SLC2A1* and *HIG2*). Other genes whose expression was effected were those involved in oxidative phosphorylation (*COX10* −1.60-fold, *CYC1* −1.70-fold and subunits of NADH dehydrogenase −1.80-fold) and the glycolysis pathway (*ALDOA* +2.15-fold, *GAPDH* +2.80-fold and *ENO1* +1.99-fold), which is consistent with data indicating that cells shift metabolic strategy of ATP production in hypoxic conditions. Cancer-associated genes linked with vascular endothelial growth factor (VEGF) angiogenesis such as *MAP2K1*, *JUN*, *ETS1* and *MMP9* were increased by +1.97, +2.22, +2.19 and +1.56-fold respectively, with $P<0.05$; and proliferation genes *RASSF1* and *CCND1* were up- and down-regulated by +1.95 and −2.04-fold respectively, $P<0.05$. These changes were validated in up to eight other parathyroid adenoma cultures. Thus, our data demonstrate that parathyroid adenomas, under hypoxic conditions, have expression of genes known to promote angiogenesis and proliferation. These results may have implications for parathyroid cell culture and parathyroid gland autotransplantation in patients.

DOI: 10.1530/endoabs.34.P406

P407

A mediastinal mass in a patient with thyrotoxicosis-recognising the association between thymic hyperplasia and Graves' disease

David Bishop[1], Vikram Lal[1], Joanne Fletcher[2], Wael Elsaify[3] & Satyajit Nag[1]
[1]Department of Diabetes and Endocrinology, James Cook University Hospital, Middlesbrough, UK;[2]Department of Radiology, James Cook University Hospital, Middlesbrough, UK;[3]Department of Surgery, James Cook University Hospital, Middlesbrough, UK.

Introduction
There is a rare but well documented association between thymic hyperplasia and Graves' disease particularly in young patients. It is important to recognise this as patients presenting with an anterior mediastinal mass will often undergo extensive investigations to exclude an underlying lymphoproliferative disorder.
Case study
A 19-year-old female with symptomatic Graves' thyrotoxicosis (TSH <0.01 mU/l; FT_4 46.8 pmol/l; TBII 5 U/l) presented with pleuritic chest pain. A CT pulmonary angiogram excluded pulmonary embolism but an incidental large anterior mediastinal soft tissue mass measuring 7.6×2.1×7.1 cm was noted. Hodgkin's lymphoma was suspected but histology from CT guided biopsy of the mediastinal mass showed a T-cell lymphoid population and features consistent with true thymic hyperplasia. PET CT showed diffuse moderate grade FDG activity within the mediastinal mass. An association between Graves' disease and thymic hyperplasia was recognised. The patient underwent an uneventful total thyroidectomy after euthyroidism was achieved with carbimazole therapy. CT scan 6 months post-surgery showed near complete resolution of the anterior mediastinal mass.
Discussion
The association between Graves' disease and thymic hyperplasia is rare but well documented.In the majority of cases, thymic enlargement is minimal, but occasionally it may be massive. The exact pathophysiology in unknown, but a postulated mechanism is the possible presence of TSH receptors in thymic epithelial cells that may be involved in the autoimmune response. Given that the differential diagnosis of an anterior mediastinal mass includes pathologies that may be managed with surgical resection for both diagnosis and treatment, recognition of the association between Graves' and thymic hyperplasia can potentially avoid unnecessary major surgery.
Treatment of Graves' hyperthyroidism usually results in complete resolution of the thymic hyperplasia.

DOI: 10.1530/endoabs.34.P407

P408

Thyrotoxic cardiomyopathy

Haval Surchi & Usha Ayyagari
Royal Berkshire NHS Foundation Trust, Reading, UK.

Introduction
We report a case of severe thyrotoxic cardiomyopathy, a potentially life threatening complication of thyrotoxicosis.
Case report
A female, age 47, usually fit and well, presented to the GP with 4 week history of feeling generally unwell, intermittent palpitations and right leg swelling.

Ultrasound ruled out a DVT. She developed shortness of breath, heat intolerance, tremor, weight loss, generalized body swelling. She noticed change in her voice and neck swelling.
On admission she had tremor, goitre, hypertension (BP 194/88 mmHg), tachycardia (HR 116). U&E and LFT were normal except for raised ALP of 196 IU/l. TFT showed TSH <0.01 mU/l, FT_4 84.1 pmol/l, TPO antibodies 405 IU/ml.
She was started on carbimazole 20 mg OD, propranolol 40 mg OD, hydrocortisone and furosemide. After endocrine review, carbimazole was increased to 60 mg OD, furosemide doubled to 80 mg OD and steroid was discontinued. Echocardiogram confirmed severe cardiomyopathy and pulmonary hypertension. Cardiology reviewed to rule out other causes of congestive heart failure. She responded to treatment and repeat TFT 7 days later showed TSH <0.02 mU/l, FT_4 23.8 pmol/l, FT_3 4.2 pmol/l.
Discussion
Thyrotoxicosis effects peripheral circulation (increased circulatory volume, reduced pre-load, pulmonary hypertension) and has direct cardiac effects including arrhythmias, alteration of myocardial contractility, left ventricular hypertrophy and cardiomyopathy.
Cardiac failure may be rate related, secondary to volume overload or left ventricular impairment. Prevalence varies from 12 to 68%. Although usually associated with underlying heart disease, it can occur in young people even in the absence of heart disease.
Dilated cardiomyopathy is reported in 1%. The mechanisms are poorly understood. It can be irreversible in up to 1/3 of patients. Risk factors include older age, male sex and duration of thyrotoxicosis. Initial management with β-blockers for rate control, diuretics and anti-thyroid medication should be followed by definitive management of thyrotoxicosis.
As a potentially reversible cause of cardiomyopathy, hyperthyroidism should be excluded in all patients.

DOI: 10.1530/endoabs.34.P408

P409

Not to worry PET, it's not cancer

Raj Tanday, Agnieszka Falinska, Tannaz Vakilgilani, YongYong Ling & Jeannie Todd
Hammersmith Hospital, London, UK.

We present two patients who have increased uptake in the thyroid on PET imaging.
Mrs AN is a 76-year-old woman with hypertension, atrial fibrillation, deep vein thrombosis and primary autoimmune hypothyroidism with positive TPO antibodies on thyroxine. When abroad she had a carotid USS which discovered a thyroid nodule. Referred to us she was euthyroid with an USS finding of a 5 mm calcified nodule with no vascularity in the right thyroid. She had a FNA in Sept 12 and Jan 13 both revealing a lymphocytic infiltrate (Thy2) consistent with Hashimoto's thyroiditis. She was reassured and discharged. She was re-referred this autumn after a private FDG PET scan was performed to investigate night sweats and palpitations. This showed high focal uptake in the thyroid and nil else. A repeat USS thyroid showed no change.
Mr NM is a 45-year-old man with a succinate dehydrogenase mutation and paragangliomas in the aorta (resected Sept 11), bilateral neck (left excised Nov 12) and primary autoimmune hypothyroidism with positive TPO antibodies on thyroxine. FDG PET imaging consistently shows increased uptake in the left thyroid as well as the expected neuroendocrine tumours. An ultrasound of the thyroid showed a bulky left lobe with a 8 mm nodule. FNA cytology confirmed lymphocytic infiltrate (Thy2) consistent with Hashimoto's thyroiditis. MRI neck showed no significant lesion within the thyroid.
Both our patients had increased uptake on FDG PET in the thyroid where there was no other clinical disease. Both these patients had treated primary autoimmune hypothyroidism with the thyroid FNA cytologies confirming a lymphocytic thyroiditis. It is important to be aware that increased uptake in the thyroid on PET maybe due to Hashimoto's thyroiditis rather than a sinister cause.

DOI: 10.1530/endoabs.34.P409

P410

PTEN hamartoma syndrome: unravelling the complexities of childhood surveillance

Harshini Katugampola, Sasha Howard & Jeremy Allgrove
Royal London Hospital, London, UK.

Background
PTEN hamartoma tumour syndrome (PHTS) is a rare autosomal dominant disorder characterised by macrocephaly and multiple hamartomas. It carries an increased risk of several cancers, including breast, thyroid and endometrium. PHTS is caused by inactivating mutations of *PTEN* (phosphate and tensin homologue deleted on chromosome 10), which encodes a tumour suppressor phosphatase. Published guidelines for surveillance are available for adult patients but not advocated in children. There is evidence of a broader spectrum of clinical features emerging making decisions surrounding appropriate paediatric surveillance and counselling highly complex.
Patients and methods
We manage two siblings with a confirmed germline truncating mutation in PTEN (R233X) inherited from their mother, who developed a compressing thyroid goitre requiring near-total thyroidectomy aged 28 years. Both children have macrocephaly and developmental delay with autistism but are otherwise asymptomatic (aged 7 and 6 years). A primary search of Medline via PubMed and secondary searches via national guideline databases were carried out.
Aim
To examine the literature for existing guidelines and epidemiological data to produce a comprehensive screening plan for our paediatric PHTS patients.
Results
12 relevant papers and two published guidelines were identified. Consensus guidelines recommend surveillance from 18 years unless there is a family history of cancer <23 years. However, a recent prospective study reported an increased risk of thyroid cancer from early childhood.
Conclusions
Available guidance for the management in childhood of PHTS patients is limited. We recommend surveillance from the point of diagnosis with annual thyroid ultrasound and targeted clinical review.

DOI: 10.1530/endoabs.34.P410

P411

A rare case of papillary thyroid cancer arising from the ovary

Venkata Katreddy, Harit Buch, Laura Pettit & Victor Oguntolu
Wolverhampton Diabetes Centre, Wolverhampton, UK.

Background
Struma ovarii, defined as containing 50% or more thyroid tissue is rare and accounts for 1% of ovarian tumours. Presentation is non-specific, relating to mass effect. Although the tumour predominantly consists of thyroidal tissue, features of hyperthyroidism occur in $<5\%$ of cases. Majority are benign, histological features of thyroid cancer are found in about 5–10%. Seventy per cent of these are papillary thyroid cancer (PTC). We present a case of struma ovarii with papillary thyroid carcinoma.
Case presentation
A 62-year-old lady presented with abdominal discomfort. Ultrasound pelvis identified a large right sided ovarian mass. CA-125 was elevated, thyroid function tests were normal. At laparotomy, a large impacted tubo-ovarian mass was identified; total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed. Histology revealed a collapsed thick walled cyst with haemorrhage. Immunocytochemistry was in keeping with papillary thyroid cancer arising in struma ovarii. Tumour was classified as FIGO 1C in view of the ruptured cyst wall.
Management
Following discussion at thyroid MDT, total thyroidectomy was recommended to facilitate adjuvant radioactive iodine ablation and exclude associated papillary thyroid cancer as described in literature. Histology of thyroid showed colloid hyperplastic nodule of 5 mm with no evidence of malignancy. Post operatively radioiodine ablation 3.7 GBq was carried out. CT and I131 scan did not identify any metastasis. Stimulated thyroglobulin was <5 (μg/l. She is currently established on levothyroxine with a view to maintaining suppressed TSH.
Discussion
This case demonstrates an uncommon clinical scenario of PTC in struma ovarii and the importance of a multidisciplinary approach in its management. Although there is no consensus on management of patients with malignant struma ovarii, our patient was risk stratified as high risk and hence surgical curative treatment of the primary tumour with total hysterectomy and bilateral salpingo-oophorectomy, followed by total thyroidectomy and radioiodine ablation.

DOI: 10.1530/endoabs.34.P411

P412

Are patients aware of the risk of agranulocytosis when taking antithyroid drugs?

Vincent Chau, Julie Andrew, Emma Ward, Ramzi Ajjan & Robert Murray
The Leeds Teaching Hospitals NHS Trust, Leeds, UK.

Background
Hyperthyroidism affects 1.3% of the population. The antithyroid drugs (ATD), carbimazole and propylthiouracil, can induce agranulocytosis, a rare but potentially life threatening side-effect with a prevalence of 0.10–0.15%. We assessed the adequacy of our patient's knowledge of this side-effect.
Methods
Patients on ATD completed a questionnaire before attending clinic. The questionnaire consisted of nine questions detailing information they had received and knowledge of how they should respond if they become unwell on ATD. Data were collected over a 3-week period.
Results
39 patients completed the questionnaire. 30 patients (77%) said they received information regarding side-effects when ATD were commenced; eight patients (21%) said they did not; and one patient (3%) was unsure. Most information was given verbally (32), and others include letters (11), cards (9) and leaflets (1). Only 19 patients (49%) patients said the information given was understandable. Most patients had not experienced side-effects (18). The most common side-effects reported were sore throat (8), lethargy, and malaise (6). When asked what they would do in the event of a sore throat, most patients (62%) said they will see their GP or attend A + E. Only seven patients (18%) knew that they needed a blood test. Most patients said they would obtain medical input immediately (36%) or same day (36%). Only 20 patients (51%) said they would stop ATD in the event of a sore throat, and 18 patients (46%) knew to restart ATD if blood test was normal.
Conclusion
This audit shows inadequate patient awareness of the potential life threatening side-effect of ATD. We have since introduced a credit card sized ATD information card for patients commencing ATD. It provides instructions for patients and healthcare professionals to follow when infections occur. We aim to re-audit 12 months after introduction of this new card to assess the impact.

DOI: 10.1530/endoabs.34.P412

P413

Myxoedema coma in a patient with bipolar disorder

Vidhya Jahagirdar, Christine May & Kristien Boelaert
University Hospitals Birmingham NHS Trust, Birmingham, West Midlands, UK.

A 63-year-old woman with a history of bipolar disorder and hypothyroidism under section 3 of the Mental Health Act in a psychiatric centre was admitted with severe hypothermia, bradycardia, hypotension and decreased GCS. She was on procyclidine and haloperidol for bipolar disorder and on intravenous antibiotics for 2 days for recurrent cellulitis in her leg. She was on levothyroxine 50 μg daily but was non compliant with her medications.
Thyroid function testing on admission revealed that she was severely hypothyroid with a serum TSH concentration of >99 mIU/l, free T_4 of 5.4 pmol/l and free T_3 of 1.5 pmol/l. Myxoedema crisis was suspected and she was admitted to ITU for ionotropic support. She was given i.v. liothyronine 50 μg followed by liothyronine 25 μg and hydrocortisone 50 μg three times daily. Oral levothyroxine 100 μg daily was commenced via nasogastric tube on day 3 and i.v. liothyronine and hydrocortisone were tapered and stopped on day 5. The clinical parameters improved and the patient was transferred to a general medical ward on day 4. The serum TSH concentration improved dramatically after administration of levothyroxine, but as the patient continued to be non compliant, she was commenced on oral levothyroxine 700 μg to be administered once a week under supervision.
Myxoedema coma is an uncommon life threatening endocrine crisis usually occurring in the elderly women, precipitated by an acute event such as infection, myocardial infarction, cold exposure, or the administration of sedative drugs. The mortality rate remains very high in these patients. Diagnosis can be delayed in patients with severe sepsis on the background of mental illness. There is no clear guideline about how these patients should be managed. Early diagnosis and management of myxoedema coma by the ITU and Endocrinologists led to a favourable outcome in our patient.

DOI: 10.1530/endoabs.34.P413

P414

Audit of thyroid surgery outcomes for benign thyroid disease at west Hertfordshire hospitals NHS trust 2010–2012

Sajini Wijetilleka, Edouard Mills, Ana Pokrajac & Julia Ostberg
West Hertfordshire Hospitals NHS Trust, Hertfordshire, UK.

Background
Surgery has a significant role in the management of benign thyroid disease, necessitating pre-operative evaluation and appreciation of possible complications. These include hypoparathyroidism and hypothyroidism. We compared outcomes of our benign thyroid disease patients requiring surgery to national standards set by the British Thyroid Association.
Method
Outcomes for patients operated between December 2010 and December 2012 with benign disease within our Trust were audited.
Results
Sixty-one patients underwent thyroid surgery (62% were physician referrals) including 54 females and seven males (mean ages 49 and 55 years respectively). Procedures performed included hemi-thyroidectomy (31/61), total thyroidectomy (13/61), subtotal thyroidectomy (15/61), completion thyroidectomy (1/61) and thyroglossal cyst excision (1/61). In the majority, surgery was indicated for clinical reasons (51/61), including multi-nodular goitre (33/61), Graves' disease (12/61), toxic nodule (3/61) and Thy3a/f disease (3/61). Pre-operative imaging was arranged in 55/61 patients. Thyroid status at the time of surgery, included euthyroid (42/61), hyperthyroid (17/61) and hypothyroid (2/61). Operative histology confirmed benign disease in 60/61 patients and one incidental focus of papillary carcinoma in the remaining patient. No early complications (within 6 post-operative months) were experienced in 35/61 patients. Early complications included hypocalcaemia (13/61), haematoma formation (4/61 with three patients requiring revision operations), wound infection (2/61), recurrent laryngeal nerve injury (7/61 although not persisting after 6 months) and hypothyroidism (12/61). Late hypocalcaemia was present in 9/61 patients, requiring treatment with calcium alone (1/9) or combined with hydroxylated vitamin D derivatives (8/9). All patients hyperthyroid pre-operatively were cured.
Conclusion
Compared with the national average, our early hypocalcaemia rate is less (21.3 vs 30%) but our late hypocalcaemia rate is greater (14.8 vs 10%). Endocrinologists and thyroid surgeons at our trust will collaborate to form a standardised care pathway for all thyroid surgery patients, to include referral to endocrinology for all patients with abnormal pre-operative thyroid function.

DOI: 10.1530/endoabs.34.P414

P415

Correlation between thyrotoxicosis and hepatic dysfunction in outpatient's endocrine clinic: a single centre experience

Victor Oguntolu, Umaira Aziz, Rolli Allain & Rosseau Gama
Royal Wolverhampton NHS Trust, Wolverhampton, UK.

Introduction
Thyrotoxicosis is a common endocrine disorder; variable degree of hepatic dysfunction is often seen with thyrotoxicosis. Severe liver dysfunction associated with thyrotoxicosis occurs rarely. The prevalence of liver abnormality with thyrotoxicosis is reported to be ~ 15–30%. The mechanism of liver abnormality is not well understood; direct thyroid hormone mediated hepatocyte injury has been raised as a possible mechanism. We conducted a retrospective review of all patients' with concomitant thyrotoxicosis and deranged liver function test who attended our endocrine clinic over 12 months period.
Methods
We reviewed all patients who attended our clinic for a period of 12 months with thyrotoxicosis. Those with abnormal LFT were selected; patients were excluded, if abnormal LFT predate the diagnosis of thyrotoxicosis, or if there is a known history of liver disease. 200 patients were diagnosed with thyrotoxicosis, 27 patients (13%) were suitable for analysis; 20 females and five males.
Results
There is female preponderance of 74%. The aetiology is: Graves' 52%; toxic goitre 19%, AIT 2%; unclear in 6%. 81% were treated with carbimazole, 19% with PTU.ALP was abnormal in 100%; in 23% both ALP and ALT were raised. Mean time to normalisation of TFT and LFT in weeks was 9.8 ± 4.6; 6.5 ± 3.6 (mean $\pm$ s.d.).The mean to normalisation of LFT depending of treatment was 6.5 ± 3.4 vs 7.4 ± 3.8 (carbimazole vs PTU). LFT normalised in all patients treated with PTU (100%).
Conclusion
We demonstrated that hepatic dysfunction is commonly associated with thyrotoxicosis (13% prevalence). The degree of abnormality does not correlate

with the severity of thyrotoxicosis. Both PTU and carbimazole are safe for the treatment of moderate liver dysfunction associated with thyrotoxicosis. There is no difference in the time to resolution between the two drugs; however PTU results in 100% resolution of liver abnormality.

DOI: 10.1530/endoabs.34.P415

P416

A case of severe hypothyroidism presenting with multi-organ failure

Eswari Chinnasamy[1,2] & Andrew Rodin[1,2]
[1]St Georges Hospital, Tooting, London, UK; [2]St Helier Hospital, Carshalton, London, UK.

Myxoedema coma or severe decompensated hypothyroidism is rare but has high mortality; appropriate early recognition and treatment is essential. We present a case of severe hypothyroidism with multi organ failure. A 58-year-old lady with several months' history of loss of appetite, tiredness and hair loss presented with progressive lethargy, sleepiness and abdominal distension for 1 week. On admission she was very drowsy, had a doughy dry skin and facial puffiness. Large ascites was present. Vital signs were stable. CXR showed left pleural effusion. Her initial biochemistry showed hyponatraemia, hypokalaemia, acute kidney injury (Cr: 2.03 μmol/l), abnormal LFTs (ALT 2898 IU/l, bilirubin 50 μmol/l), early disseminated intravascular coagulation picture and type 2 respiratory failure (pH: 7.09, pCO2 12) Full liver screens including paracetamol level were negative. Supportive treatments including BiPAP were commenced. Subsequently her TSH was 63 mU/l with undetectable FT_4. She was commenced on levothyroxine 25 μg daily along with intravenous hydrocortisone. She remained on BiPAP without improvement for 4 days. Liothyronine 10 μg tds was added, levothyroxine dose increased to 75 μg od and she improved significantly over the next 2 days. She was off BiPAP, AKI resolved and LFTs improved significantly. USG confirmed significant ascites without evidence of cirrhosis or portal vein thrombosis. Diagnostic tap confirmed this to be a transudate with no growth or malignant cells. Echocardiogram was normal. Gradually her LFTs normalised. Her synacthen test was normal and the hydrocortisone was stopped. 6 weeks later there was complete resolution of ascites, pleural effusion and skin has normalised.

Discussion

Hypothyroidism needs to be considered in anyone with unexplained type 2 respiratory failure. Optimal replacement of thyroid hormone in this condition remains under debate.

DOI: 10.1530/endoabs.34.P416

P417

Alemtuzumab and thyroid dysfunction in patients with multiple sclerosis: experience in a university hospital

Maneesh Udiawar & Hemanth Bolusani
Centre for Endocrine and Diabetes Sciences, Cardiff University, Cardiff, UK.

Objective

The MAB, alemtuzumab has been demonstrated to reduce the relapse and the risk of accumulation of sustained disability in multiple sclerosis when compared to β-interferon. The development of autoimmune diseases, including thyroid disease has been reported in the literature with a frequency of 15–25%.

Methods

We reviewed the case notes of 39 patients with multiple sclerosis initiated on alemtuzumab in our hospital from 2006 to 2012 and studied the clinical course of the patients who subsequently developed thyroid dysfunction.

Results

Of the 38 patients reviewed (25 females and 13 males) 15 patients (40%) developed thyroid dysfunction (ten females and five males). Nine patients developed symptoms of thyrotoxicosis (seven females and two males) five patients developed hypothyroidism (three males and two females) and one patient initially developed hypothyroidism and was commenced on thyroxine replacement and subsequently developed symptoms of thyrotoxicosis after withdrawal of thyroxine. Of the thyrotoxic patients ($n=10$) seven patients had TRAB positive antibodies (70%). The mean duration of development of abnormal thyroid function tests was 31 months after administration of 1st cycle of alemtuzumab. All patients with thyrotoxicosis were started initially on antithyroid medications and on further relapse four patients were offered radioiodine therapy with complete resolution and LT_4 replacement for post RAI hypothyroidism.

Conclusion

Graves' disease is a common complication of alemtuzumab use. The mechanism of thyroid autoimmunity after alemtuzumab treatment is likely related to the loss of self tolerance in the homeostatic proliferation which occurs following profound lymphopenia. Ophthalmopathy may occur in some patients. Close monitoring of serum TSH levels is essential and an early response to a relative fall in levels that remain within the normal range in the form of more frequent monitoring is warranted. Monitoring must be maintained for many years.

DOI: 10.1530/endoabs.34.P417

P418

Consequences of an inadvertent iodine-rich diet, in previously definitively treated Graves' disease

Rebecca Shankland
Countess of Chester Hospital NHS Foundation Trust, Countess of Chester Health Park, Liverpool Road, Chester CH2 1UL, UK.

Case report

A 41-year-old lady, previously diagnosed with Graves' disease, had been treated with 12 months carbimazole in a standard block and replace regimen. The hyperthyroidism relapsed following treatment withdrawal so she was treated definitively with radio-iodine therapy, remaining well for 5 years, without hypothyroidism. The patient commenced the lighter life diet due to weight gain and subsequently presented with weight loss of a stone and atrial fibrillation (AF). She was found to have relapsed hyperthyroidism (FT_4 45 pmol/l, TSH suppressed.) The lighter life diet involves combined group counselling sessions and a very low calorie diet (VLCD) constituting pre-prepared food packs (shakes and mousses). The food supplements are documented to contain iodine, between 90 and 117.5 μg/100 g dependant on the snack or meal used. (∼110 μg/day). On admission, serum iodine levels were elevated at 0.93 μmol/l (0.32–0.63).

Discussion

Iodine has a central role in thyroid hormone synthesis (recommended daily intake of 50 μg). Whilst both iodine deficiency and excess can cause thyroid dysfunction, iodine-induced hyperthyroidism (IIH) is commoner in areas of iodine deficiency or in patients with pre-existing thyroid disease. IIH is usually self-limiting (lasting 1–18 months) if the source of iodine is eliminated but thionamide treatment may speed recovery. Other sources of inadvertent iodine supplementation include multivitamin preparations, cough medicines and iodinated contrast media used in CT scanning/angiography. Detailed history of potential iodine ingestion is required in all hyperthyroid patients.

Outcome

The patient was treated with β blockers for the AF, which settled, and started with titratable dose carbimazole which was discontinued after 6 months. She remains euthyroid 6 months off treatment (and off iodine supplementation).

DOI: 10.1530/endoabs.34.P418

P419

A case of myxoedema coma and malnutrition

Alexander Tattersall, Rajni Mahto, Rashmi Manjunatha & Jack Storrow
South Warwickshire Foundation Trust, Warwick, UK.

Introduction

Myxoedema coma is a rare complication of hypothyroidism. Although malnutrition is considered rare in developed countries, research has indicated that there are more than three million people in the UK suffering from or at risk of malnutrition. We present a case of myxoedema coma co-existing with malnutrition.

Case report

A 51-year-old Caucasian female presented with acute confusion, peripheral oedema and a bilateral lower limb rash. On examination she exhibited proximal muscle weakness, slow relaxing reflexes and an erythematous macular rash on her lower legs. She had a family history of hypothyroidism. Investigations revealed severe hypothyroidism (TSH >100 mU/l and T_4 6.6 pmol/l), a normocytic anaemia (Hb 9.1 g/dl) and hyponatraemia (Na 114 mmol/l). A Short Synacthen test was performed with an adequate response and both a coeliac screen and vitamin B12 levels were normal. An ultrasound scan of the thyroid revealed features consistent with Hashimoto's thyroiditis and thyroid peroxidase antibody was positive. She was treated with levothyroxine 100 μg once daily. Punch biopsies of the skin rash were suggestive of zinc deficiency. Further investigations revealed deficiencies in magnesium and vitamin D as well as hypoalbuminaemia (27 g/l). She had a BMI of 15. In view of these findings, a diagnosis of

malnutrition was reached. The patient was treated with vitamin D, zinc and dietary supplements to good effect. Her sodium normalised, her confusion resolved and her muscle weakness improved.

Discussion

Primary hypothyroidism presenting with myxoedema coma is an important differential diagnosis in all patients presenting with acute confusion. Timely diagnosis can be life-saving. This case highlights the importance of realising that not all patients with hypothyroidism will exhibit classical features such as weight gain.

DOI: 10.1530/endoabs.34.P419

P420

Is there a difference in observed bone mineral density at diagnosis of overt or subclinical thyrotoxicosis?

Cathy McHugh[1] & Paul Hession[2]
[1]Sligo Regional Hospital, Sligo, Ireland; [2]National University of Galway, Sligo, Ireland.

Introduction

Early thyrotoxicosis is associated reduced bone density. The aim of this study is to determine any difference between bone mineral density (BMD) in those presenting with overt or subclinical thyrotoxicosis.

Methods

Retrospective observational study of BMD in individuals presenting with thyrotoxicosis from 2008 to 2013. BMD was assessed by bone densiometry using *T*, *Z*, and total BMD within 1 year of first abnormal thyroid function tests.

Results

91 people were included: 64 women and 27 men. 49 had overt thyrotoxicosis at diagnosis ($n=15$ aged 20–50 years, $n=34$ aged >50 years), 40 had subclinical thyrotoxicosis (five aged 20–50 years, 35 aged >50 years). The median age was 43 years (overt), 42 years (subclinical) aged 20–50 years, 58.5 (overt), 70 years (subclinical) in the >50 years group.

In those aged 20–40 years the mean TSH at diagnosis ($n=20$) was 0.03 ± 0.02 U/ml, fT_4 27.17 ± 2.5 pmol/l, and in the >50 years age ($n=69$) mean TSH was 0.16 ± 0.04 pmol/l, fT_4 21.46 ± 1.34 pmol/l. There was no difference in BMD, *T* or *Z* scores in overt or thyrotoxic patients in any of the age ranges.

In the 20–50 years age group 4 had a *Z* score <-2.5, two in L1L4 and two femoral neck (all subclinical). 12 had *Z* scores between -2.5 and -1.0 (two in L1L4 (two overt) and ten femoral neck (seven overt, three subclinical). Aged >50 years 30 had *T* scores <-2.5 (L1L4(ten overt, six subclinical) 12 femoral neck (six overt, six subclinical), two radius (subclinical), 84 had *T* scores -1.0 to -2.5 (L1L4 (seven overt, 12 subclinical), 64 femoral neck (35 overt, 29 subclinical) and one radius(subclinical).

Conclusion

There is no difference in *Z* score and *T* score between those who presented with overt thyrotoxicosis and those with subclinical thyrotoxicosis. There were a number with *Z* scores -2.5 to -1.0 which merit rescanning but overall the prevalence of lower *T* scores in those aged >50 years presenting with thyrotoxicosis was high and this was their first DEXA. This study highlights the importance of DEXA scanning in this population.

DOI: 10.1530/endoabs.34.P420

P421

Treatment with a TRα1 antagonist increases bone mineral content

Julian A Waung, J H Duncan Bassett & Graham R Williams
Imperial College London, London, UK.

Thyroid hormones regulate adult bone turnover. Thyrotoxicosis results in high turnover osteoporosis whilst hypothyroidism leads to low bone turnover with increased bone mass and mineralisation. T_3-target tissues express thyroid hormone receptor alpha (TRα), thyroid hormone receptor beta (TRβ)or both receptors. TRα1 mediates the actions of T_3 in bone and in skeletal cells TRα1 mRNA expression is 12-fold higher than TRβ1. Accordingly, adult mice lacking TRα ($TR\alpha^{0/0}$) have reduced bone turnover and increased bone mass. Dronedarone is a non-iodinated amiodarone derivative, reported to act as a TRα1 antagonist. We hypothesised, therefore, that treatment with dronedarone would increase bone mass and strength in adult mice.

To investigate this hypothesis, 9-week-old male C57Bl/6 WT mice were treated with vehicle or dronedarone 60 mg/kg per day for 17 weeks until sacrifice. Femurs and vertebrae were analysed by digital X-ray microradiography to determine bone mineral content and mid-diaphyseal cortical thickness. The bio mechanical properties of long bones were quantified by destructive three point bend testing.

Femoral bone mineral content was increased in dronedarone treated mice (Kolmogorov-Smirnov test; $P<0.01$, $n=12$) but vertebral bone mineral content was unaffected. Furthermore, a trend towards increased cortical thickness was observed in dronedarone treated animals (Student's *t*-test; vehicle 205 ± 10 μm, dronedarone 220 ± 14 μm, $P=0.06$, $n=12$). Nevertheless, mechanical testing of bone strength revealed that yield load, maximum load, fracture load and stiffness did not differ in dronedarone treated mice.

These studies demonstrate treatment of adult mice with dronedarone increases bone mineral content. Further detailed and comprehensive skeletal analysis is now required to evaluate the potential of TRα1 antagonism as a novel therapeutic approach in the treatment of osteoporosis.

DOI: 10.1530/endoabs.34.P421

P422

Neutrophil phagocytic capacity is lower in patients with abnormal thyroid function in critical illness

Emma Browne, John Simpson & Salman Razvi
Newcastle University, Newcastle upon Tyne, UK.

Background

Thyroid function is affected by critical illness – termed as non-thyroidal illness – and usually presents with low serum TSH and FT_3 levels but normal FT_3 concentrations. Critically ill patients may also have impaired immune function that could contribute to increased susceptibility to acquired infections and mortality. Thyroid hormones influence neutrophil levels and function but the effect of non-thyroidal illness on neutrophil function in critically ill patients is unknown.

Methods

Critically ill patients ($n=24$) admitted to three Intensive Care Units (ICU) were studied. Blood samples for assessment of thyroid function and neutrophil phagocytic activity were obtained on a single day within 72 h of admission. Neutrophils were isolated from whole blood using a percoll gradient separation technique and incubated with zymosan on a 24 well plate. Light microscopy was used to determine the percentage of neutrophils ingesting ≥2 zymosan particles. Severity of illness was assessed by APACHE II scores.

Results

Prevalence of abnormal thyroid function in critically ill patients was 79.2% (19/24): isolated low FT_3 syndrome 38% (9/24), overt hyperthyroidism (1), overt hypothyroidism (1), subclinical hyperthyroidism (4/24) and subclinical hypothyroidism (2/24) whereas one patient each had low TSH, FT_4 and FT_3 and normal TSH with low FT_4 and FT_3 levels, respectively. Neutrophil phagocytic capacity was significantly lower in the abnormal thyroid function group compared to those with normal function, 38.9 vs 53.7%; $P=0.03$. No association was found between phagocytosis and any single thyroid function parameter or with severity of illness. However, day of sampling after admission was found to be a significant confounder.

Conclusions

Abnormal thyroid function is common within the first 72 h after admission to ICU with low FT_3 syndrome being the dominant picture. Patients with abnormal thyroid function have lower neutrophil phagocytic capacity than those with normal thyroid function but this is likely to be influenced by duration of illness. These results need to be confirmed in a larger sample and at varying time points after admission.

DOI: 10.1530/endoabs.34.P422

P423

Autoimmune thyroid disease in the presence of resistance to thyroid hormone or TSH-secreting pituitary tumour: a diagnostic challenge

Carla Moran, Olympia Koulouri, Fleur Talbot, Catherine Mitchell, Nadia Schoenmakers, Greta Lyons, Mark Gurnell & V K K Chatterjee
Wellcome Trust-MRC Institute of Metabolic Science, University of Cambridge, Cambridge, UK.

Background

Hyperthyroxinaemia with non-suppressed TSH, due to resistance to thyroid hormone (RTH) or TSH-secreting pituitary tumour (TSHoma), can be difficult to diagnose, particularly with coincident autoimmune thyroid disease (AITD).

Methods
To determine presentation patterns of AITD coincident with RTH or TSHoma, we analysed our cohort of cases with dual diagnoses.
Results
Nine patients with RTH had AITD. Six had Graves' disease (GD); RTH was suspected when anti-thyroid drug treatment led to unexpected TSH increase despite hyperthyroxinaemia (e.g. initial TSH <0.03 mU/l, TT_4 300 nmol/l; post carbimazole TSH 6 mU/l, TT_4 150 nmol/l (NR 50–150)) or when hyperthyroxinaemia was associated with incompletely suppressed TSH (e.g. FT_4 89.6 pmol/l, FT_3 26.7 pmol/l, TSH 0.04 mU/l during GD flare-up, with baseline TSH 1.7 mU/l, FT_4 27.9 pmol/l, FT_3 16.7 pmol/l). Three had autoimmune hypothyroidism with elevated TSH levels but high-normal TH (e.g. TSH 29.8 mU/l, FT_4 20.8 pmol/l, FT_3 7.2 pmol/l) and positive anti-TPO antibody titres.
Two patients with TSHoma had coincident AITD; the first presented with an elevated TSH and concomitant mild hyperthyroxinaemia (TSH 35.2 mU/l, FT_4 22.2 pmol/l), the second presented with failure to suppress TSH after levothyroxine for presumed primary hypothyroidism (TSH 5.9 mU/l, FT_4 25 pmol/l, on 50 μg of levothyroxine). Both had raised TPO antibody titres.
20% of our genetically heterogeneous RTH cohort ($n=160$) without known AITD, are anti-TPO antibody positive, but all are anti-TSHR antibody negative.
Conclusion
Resistance within the HPT axis (RTH) or inappropriate TSH secretion (TSHoma) should be suspected in i) autoimmune hypothyroidism where physiological dose thyroxine replacement fails to correct elevated TSH, ii) thyrotoxic patients with incompletely suppressed TSH especially when normalisation of TH levels in response to anti-thyroid drug treatment results in exaggerated TSH rise and iii) patients with elevated TSH but high-normal TH concentrations. Lastly, the increased prevalence of positive thyroid autoantibodies in RTH suggests these patients may be at greater risk of developing AITD.

DOI: 10.1530/endoabs.34.P423

P424

Evaluation of calcitonin as a screening tool for medullary thyroid carcinoma in patients with nodular thyroid disease

Sivasujan Sivasubramaniyam, Roy Sherwood & Jackie Gilbert
King's College Hospital, London, UK.

Background and aims
The use of serum calcitonin to screen patients with nodular thyroid disease for medullary thyroid cancer (MTC) remains controversial due to conflicting data regarding its sensitivity, specificity and cost effectiveness. Currently, the use of calcitonin is recommended by the European consensus group but not the American Thyroid Association. We aimed to formally evaluate the utility of calcitonin as a screening tool for MTC in patients presenting to the thyroid nodule clinic at our institution.
Methods
Patients referred to a thyroid nodule clinic in a tertiary centre between 1st January 2010 and 31st July 2013 were evaluated using serum calcitonin, TPO antibodies and TFTs with cytology/histology and radiology assessment. Exclusion criteria included known MTC or positive MEN status. Calcitonin was deemed elevated if $\geq$4.8 ng/ml for females and $\geq$11.8 ng/ml for males.
Results
302 patients (age: 49.8 ($\pm$15.7) years; female: 84.1%) were included in the audit. 20 patients had an elevated calcitonin of which two were confirmed to have MTC. 12 cases had no features of malignancy on cytological and/or histological evaluation. Three cases remain under investigation and three cases were lost to follow-up. MTC accounted for 13.3% of all thyroid malignancies detected.
Conclusion
Our results revealed that in patients referred for evaluation of thyroid nodular disease, 6.6% had an elevated calcitonin and 10% of those elevated values were associated with a new diagnosis of MTC. This is in keeping with other reported studies (10–40%). Both cases of MTC had abnormal radiological and cytological findings. Calcitonin screening did not identify any cases of MTC with otherwise normal or indeterminate parameters. Although case numbers were low, it is uncertain whether calcitonin provides added value as a screening tool for MTC in thyroid nodular disease.

DOI: 10.1530/endoabs.34.P424

P425

RET mutation negative familial medullary thyroid carcinoma: four families and literature review

Donato Iacovazzo[1,4], Patrick Morrison[2], William Foulkes[3], Douglas Ross[6], Francesca Lugli[4], Plamena Gabrovska[1], Emanuela Lucci-Cordisco[5], Giovanni Neri[5], Laura De Marinis[4] & Marta Korbonits[1]
[1]Centre for Endocrinology, William Harvey Research Institute, Queen Mary University of London, London, UK; [2]Regional Medical Genetics Centre, Belfast City Hospital, Belfast, UK; [3]Departments of Medicine, Human Genetics and Oncology, McGill University, Montreal, Quebec, Canada; [4]Division of Endocrinology, Universita' Cattolica del Sacro Cuore, Rome, Italy; [5]Institute of Medical Genetics, Universita' Cattolica del Sacro Cuore, Rome, Italy; [6]Department of Endocrinology, Harvard Medical School, Boston, Massachusetts, USA.

Approximately 25% of the reported cases of MTC are familial. Familial MTC can occur as part of MEN2-syndrome or as familial MTC alone (fMTC) defined as more than ten carriers in the kindred, or multiple carriers or affected members over the age of 50 with an adequate medical history excluding pheochromocytoma. The vast majority of MEN2 families (98%), as well as fMTC kindreds (88%) harbour a RET mutation. In MEN2A, mutations at codon-634 (exon-11) account for 85% of all mutations so far identified; mutations at codons-609, 611, 618, 620 (within exon-10) account for further cases. In MEN2B, about 95% of patients carry the M918T (exon-16). The 39 RET germline mutations identified in fMTC patients are all missense changes in exons-5, 8, 10, 11, 13, 14, 15, 16, except for a 9-bp duplication after codon-531. About 7% of sporadic MTC cases carry a RET mutation. Somatic RET mutations in sporadic MTC tumour samples have been identified in 40–50% of cases, the most common being M918T. There are very few reports on RET mutation-negative fMTC. An Italian study analysed 250 families with hereditary MTC and six families (2.4%) were RET mutation negative. Here, we report four families with RET negative fMTC: an Italian family with 11 members, a family from Northern Ireland with five affected members, an Italian-Argentinian family with two affected subjects and a Greek family with five affected. Several affected members in these families underwent genetic testing, but no RET mutations have been found at direct sequencing of the whole-coding sequence. An autosomal dominant inheritance pattern was observed together with high penetrance. In the Italian family the mean age at diagnosis is 25.8 years (s.d. 7.8) and interestingly none of the patients have distant metastasis, suggesting a possibly better prognosis compared to RET-related fMTC. Identification of the disease causing mutations in these families might reveal a novel pathway and drug target in MTC.

DOI: 10.1530/endoabs.34.P425

P426

Small papillary thyroid carcinoma: a 'benign' condition?

Ruxandra Dobrescu[1], Dumitru Ioachim[1], Anca Simioniuc[2], Corin Badiu[1,2] & Andrei Goldstein[1]
[1]CI Parhon National Institute of Endocrinology, Bucharest, Romania; [2]Carol Davila University of Medicine and Pharmacy, Bucharest, Romania.

Often discovered incidentally, small foci ($\leq$2 cm) of papillary thyroid carcinomas (PTC) are its most frequent presentation. Despite favourable prognosis, the extent of surgery and use of radioablation is still controversial.
Objective
Prospective cohort study of all patients admitted between 2007 and 2011, for thyroid surgery in the Institute of Endocrinology, Bucharest, Romania, and had pT1a or pT1b PTC.
Patients and methods
We identified 109 patients who fulfilled the criteria (92 women and 17 men), aged 46.63$\pm$13.81 years. In most patients PTC was discovered incidentally after thyroid surgery; only 20.7% had previous suspicious FNAB. In 87.21%, total thyroidectomy was performed *per primam*; 16 patients had initial partial thyroidectomy and later completion thyroidectomy. Tumour diameter was 0.77$\pm$0.59 cm; 70.64% were T1a and 33.94% were multifocal (additional foci found in 9/16 patients with completion thyroidectomy). After surgery the patients were followed-up with serial ultrasound and stimulated thyroglobulin (STgl).
Results
Post-op STgl was 1.94$\pm$3.39 ng/ml; 71 (68.27%) had STgl levels $<$2 ng/ml, while in 33 (31.73%), STgl$\geq$2 ng/ml (range, 2–28.4). Radioablation was performed in 61 patients: 44.16% T1a vs 84.37% T1b, $P=0.0001$. The total dose was significantly higher for patients treated between 2007 and 2009 vs 2010 and 2011: 110.33$\pm$43.85 vs 75.87$\pm$36.22 mCi, $P=0.01$. Whole body scan was performed in 56/109 patients, being positive in 2 (3.56%). At the end of follow-up (23.90$\pm$14.13 months), ultrasound showed small thyroid remnants in 8.42%.

The last STgl levels were <2 ng/ml in 74 (74.7%) patients, with no significant differences T1a vs T1b or multicentric vs unicentric PTC. The rest had higher STgl levels (range, 2–10.16 ng/ml) probably due to thyroid remnants/low volume residual tumour. Lower STgl levels (P=NS) were noted in radioablated vs nonablated patients (0.33 ± 0.32 vs 2.23 ± 3.24 ng/ml).
Conclusion
Small PTC often has an excellent prognosis. For un-ablated patients, a long term follow-up is required for the STgl trend to reveal recurrences and allow appropriate management.
DOI: 10.1530/endoabs.34.P426

P427

Renal function in goitrous patients attending endocrinology clinic in a South Western Nigerian hospital
Olubukola Ojo[1,3], Olalekan Ojo[1,3] & Rosemary Ikem[2,3]
[1]Federal Medical Centre, Owo, Ondo, Nigeria; [2]Obafemi Awolowo University, Ile-Ife, Osun, Nigeria; [3]University College Hospital, Ibadan, Oyo, Nigeria.

Introduction
Thyroid disorders are the second most common endocrine disorders after diabetes mellitus. These disorders are associated with cardiovascular complications which increase morbidity and mortality in affected patients. The kidney normally plays an important role in the metabolism, degradation, and excretion of several thyroid hormones. The study sets out determine renal function in patients with goiter.
Methodology
The study design was cross sectional involving 100 subjects with goitre presenting to the Endocrinology Clinic of OAUTHC. The presence of thyroid dysfunction was assessed from clinical history, clinical examination and confirmed with biochemical tests. Electrolytes, urea, and creatinine were done in all subjects and the estimated glomerular filtration rate (eGFR) estimated using modification of diet in renal disease formula.
Results
The mean ($\pm$s.d.) age for subjects in the study population was 44.6 ± 13.8 years. Majority (47.3%) of subjects in the study population were in the age group 40–59 years. Twelve (12%) subjects with goitre were males while 88 (88%) were females giving a female : male ratio of 7.3:1. A positive correlation was established between free triiodothyronine and estimated glomerular filtration rate ($r=0.531$, $P=0.063$) and free thyroxine and estimated glomerular filtration rate($r=0.897$, $P=0.013$). A negative correlation was observed between sensitive TSH and estimated glomerular filtration rate but this was not statistically significant ($r=-0.189$, $P=0.060$)
Conclusions
Declining renal function could be an indication of hyperthyroidism in patients with goiter.
Key Words: goiter, renal function, thyroxine.
DOI: 10.1530/endoabs.34.P427

P428

The influence of thyrotoxicosis on circulating cortisol and response to Synacthen
Alex Chowdhury[1,2], Aziza Khanom[1,2], Tomas Agustsson[1], Sunita Sandhu[1], Arun Sankaralingam[1], Louise Breen[1], Jake Powrie[1], Stephen Thomas[1] & Barbara McGowan[1]
[1]Guy's and St Thomas' NHS Foundation Trust, London, UK; [2]King's College London, London, UK.

Background
It is recognised that there is 'cross-talk' between the thyroid and hypothalamo-pituitary–adrenal (HPA) axes. The few available studies suggest that cortisol levels may be low and cortisol metabolism altered in some patients with thyrotoxicosis.
Aim
To compare HPA axis variables in the thyrotoxic state (TT) and euthyroid state (EU).
Materials and methods
Short synacthen testing (250 μg tetracosactide) was conducted on eight adults subjects with Graves' disease when thyrotoxic (elevated free thyroid hormones and TSH <0.01 mU/l) and subsequently repeated after achievement of EU (normal range TSH). At each assessment measurements included: total plasma cortisol and salivary free cortisol at 0, 30, and 60 min following a 0900 h Synacthen; plasma ACTH and cortisol binding globulin (CBG) were measured at $t=0$, and a 24 h urine free cortisol collected.
Results
In TT, total basal plasma cortisol was significantly lower compared to patients in EU (241.0 ± 49.4 vs 415.0 ± 60.7 nmol/l, TT vs EU, $P<0.01$). Similarly, peak response to synacthen was reduced (688.6 ± 55.6 vs 899.4 ± 88.4 nmol/l, TT vs EU, $P<0.01$). ACTH values were similar in both states but baseline salivary free cortisol was lower in TT (5.2 ± 0.8 vs 16.4 ± 1.4 nmol/l, TT vs EU, $P< 0.05$), but not peak salivary free cortisol post Synacthen (48.0 ± 3.9 vs 45.6 ± 6.4 nmol/l, TT vs EU, $P=0.4$). CBG was lower in TT (39.7 ± 5.4 vs 56.6 ± 14.4 mg/l, TT vs EU, $P=0.093$). There was no significant difference in urinary free cortisol between TT and EU.
Discussion
In this small study, basal and stimulated levels of total cortisol were reduced in the TT state, which may be explained by a reduction in CBG levels. However, basal salivary cortisol was significantly reduced in TT with preservation of stimulated response. Further studies are required to determine the clinical consequences of these changes and understand whether cortisol production and utilisation are altered in TT.
DOI: 10.1530/endoabs.34.P428

P429

Frequency of biochemical thyroid dysfunction in hospitalised patients: analysis of 280 000 admissions to a large centre
Barbara Torlinska, Jamie Coleman, Mariam Afzal, Jayne Franklyn & Kristien Boelaert
University of Birmingham, Birmingham, UK.

Routine thyroid function testing (TFT) in hospitalised patients is not recommended; however, delayed management of thyroid dysfunction may have significant consequences. We quantified the rate of TFT in hospitalised patients and identified factors influencing the likelihood of finding significantly abnormal TSH (<0.01 or >10 mUI). TFTs were performed during 26 937/280 000 (9.6%) admissions between 2007 and 2011. 57.6% of those tested were female and mean age was 63.8 ($\pm$19.3 s.d.) years. 75% were emergency admissions and the median hospital stay was 7 days. The primary reason for admission included circulatory (24.2%), respiratory (17.3%), digestive (12.3%), cancer (9.2%), and endocrine disorders (4.4%). 15.0% of those tested were undergoing active treatment for hyper-or hypothyroidism. Multivariate regression analysis identified current 'thyroid' treatment (AOR=3.72), female gender (AOR=1.41), longer hospital stay (AOR=1.48 2–4 days; AOR=2.22 5–10 days; AOR=5.00 >10 days vs 1day), older age (AOR=1.40, 1.68, 1.66, and 2.12 per quintile) and emergency admission (AOR=1.18 vs elective) as independent factors associated with increased probability of testing. Those with endocrine disorders were more likely (AOR=2.36) and those with neoplasms (AOR=0.43) or digestive disorders (AOR=0.65) less likely to undergo thyroid function testing compared with subjects with circulatory diseases. Significant TSH abnormalities were found in 1481 patients (5.9% of those tested). Subjects with a primary cancer or an endocrine diagnosis (AOR=3.79 and AOR=2.19 vs circulatory disorders), females (AOR=1.93), those not undergoing active 'thyroid' treatment (AOR=4.0 vs active treatment) and those admitted for elective procedures (AOR=1.4 vs emergency) all had a higher likelihood of having significantly abnormal TSH concentrations.
Conclusions
Within a very large cohort, fewer than 10% of hospitalised patients had thyroid function tested during their in-patient stay; abnormal TSH concentrations were evident in only a small proportion. Further studies are required to identify appropriate drivers for thyroid function testing during hospitalisation to best identify those with thyroid dysfunction who would benefit from further investigation and treatment.
DOI: 10.1530/endoabs.34.P429

P430

Pre-operative cytology vs post-operative histology in thyroid nodules
Gurmit Gill, Paul Wilson, Karthik Kalyanasundaram, George Varughese & Lakshminarayanan Varadhan
University Hospitals North Staffordshire NHS Trust, Stoke-on-Trent, UK.

Aim
The aim of our study was to assess the correlation between pre-operative fine needles aspiration based cytology with post-operative histology in patients with thyroid nodules.

Methods
A retrospective analysis of patients who had thyroidectomy (hemi, subtotal, or total) was conducted and information on histology was obtained. Pre-operative FNA (done once or twice) was also obtained and compared with histology. Patients operated for Graves' disease or completion thyroidectomy were excluded from analysis.
Results
334 patients were identified – total thyroidectomy 36, subtotal 30, lobectomy/hemithyroidectomy 252, and other type of surgeries 16.28% ($n=95$) were malignant lesions.
One Pre-op Cytology:
Thy1 ($N=96$): Cyst 1%; benign nodule 84%; malignant 15%.
Thy1c ($n=23$): Cyst 4%: benign 78%; malignant 18%.
Thy2 ($n=64$): Cyst 3%; benign 86% and malignant 11%.
Thy3 ($n=110$): benign 67%; malignant 33%.
Thy4/5 ($n=41$): Cyst 2%, benign 15%, and malignant 83%.
In patients with malignancy, cytology was: Thy1 19%, Thy2 7%, Thy3 38%, and Thy4/5 36%.
Two Pre-op cytology:
59 patients had two FNAs done preceeding surgery.
Malignancy on histology was present in 14; of these, only 2 (14%) had at least one cytology of Thy4 or above (14%); 11 patients (79%) had at least one Thy3 or above; 3 (21%) had Thy1 on both occasions
Conclusion
There is significant discordance between cytology and histology in patients with thyroid nodule. Single Thy1 samples need to be investigated due to the risk of undiagnosed malignancy. Single cytology sample may miss 26% and even double cytology could miss 21%. A multi-disciplinary approach supported by multi-faceted evidence from history, clinical examination, radiology, and histopathology is required in patients with thyroid nodules.

DOI: 10.1530/endoabs.34.P430

Author Index