TABLE OF CONTENTS

ESA OFFICE BEARERS 2010

President	Mark McLean
President Elect	Vicki Clifton
Secretary	David Phillips
Treasurer	Bu Beng Yeap
Councillors	Tim Cole
	Evan Simpson
	Peter Ebeling
	David Healy
	Warwick Inder
	Helena Teede
	Leon Bach

PAST ESA OFFICE BEARERS 1958-2010

DATE	PRESIDENT	VICE PRESIDENT	SECRETARY	TREASURER
1958-60	E.Downie		P.Taft	P.Taft
1960-62	C.W.Emmens		K.Harrison	K.Harrison
1962-64	K.Harrison		I.Thomas	I.Thomas
	C.W.Emmens (March 63)			
1964-66	B.Hetzel	V.Trikojus	I.Jarrett	I.Jarrett
1966-68	B.Hudson	V.Trikojus	R.Melick	I.Jarrett
1968-70	P.Taft	R.Cox	R.Melick	I.Jarrett
1970-72	I.Jarrett	K.Ferguson	T.J.Martin	L.Lazarus
1972-74	K.Ferguson	L.Lazarus	R.D.Gordon	L.Lazarus
1974-76	H.G.Burger	J.R.Turtle	S.Posen	C.J.Eastman
1976-78	S.Posen	J.P.Coghlan	P.E.Harding	C.J.Eastman
1978-80	J.P.Coghlan	C.J.Eastman	R.G.Larkins	J.W.Funder
1980-82	C.J.Eastman	J.W.Funder	D.P.Cameron	G.L.Warne
1982-84	J.W.Funder	R.G.Larkins	R.C.Baxter	G.L.Warne
1984-86	R.G.Larkins	D.P.Cameron	R.C.Baxter	D.M.Hurley
1986-88	D.P.Cameron	R.C.Baxter	S.J.Judd	D.M.Hurley
1988-90	R.C.Baxter	S.J.Judd	J.R.Stockigt	D.J.Handelsman
1990-92	J.R.Stockigt	J.A.Eisman	G.W.Tregear	D.J.Handelsman
1992-94D.	J.Handelsman	P.J.Fuller	R.L.Prince	D.J.Topliss
1994-96	P.J.Fuller	R.L.Prince	G.P.Risbridger	D.J.Topliss
1996-98	D.J.Topliss	R.J.Rodgers	G.P.Risbridger	M.S.Lewitt
1998-00	R.J.Rodgers	J.D.Zajac	K.K.Y.Ho	M.S.Lewitt
				B.J.Waddell (May 99)
2000-02	K.K.Y.Ho	B.J.Waddell	B.Canny	C.Coulter
2002-03	B.Canny	J.D.Zajac	R. Cuneo	C.Coulter
2004-06	J.D.Zajac	L.Bach	M.McLean	V.Clifton
2006-08	L. Bach	M. McLean	D. Phillips	V. Clifton
2009-10	M. McLean	V. Clifton	D. Phillips	B. Yeap
				V. Clifton (Feb 10)

SOCIETY SECRETARIAT - ENDOCRINE SOCIETY OF AUSTRALIA

Ivone Johnson
145 Macquarie Street
SYDNEY NSW 2000
Ph: 02 9256 5405 Fax: 02 9251 8174
Email: esa@racp.edu.au
Website: www.endocrinesociety.org.au

SPONSORS OF THE ENDOCRINE SOCIETY OF AUSTRALIA

ESA AWARD SPONSORS

FUTURE MEETINGS

ESA Seminar
29th April – 1st May 2011
Destination to be announced
www.esaseminar.org.au

ESA Clinical Weekend
26th – 28th August 2011
Western Australia
www.esaclinicalweekend.org.au

ESA/APEG Combined Annual Scientific Meeting
28h August – 31st August 2011
Perth Convention Centre
www.esa-srb.org.au

CONFERENCE ORGANISING COMMITTEES

The Local Organising Committee
Convenor: Ros Bathgate
Charles Allen (SRB), Mike Bertholdo, Simon de Graaf, Eva Dimitriadis, Kathryn Gatford (ESA), Chris Grupen (SRB), Sue Lynn Lau, Eileen McLaughlin (SRB), Kirsty Walters

ESA Program Organising Committee
Kathy Gatford (chair), Morton Burt, Lisa Butler, Jui Ho, Beverly Muhlhausler, David Torpy
The Program Committee would also like to acknowledge the assistance with reviewing abstracts provided by Tina Bianco-Miotto and Ian Chapman.

Conference Secretariat
ASN Events Pty Ltd
3056 Frankston-Flinders Road, (PO Box 200)
BALNARRING VIC 3926
Phone: 03 5983 2400 Fax: 03 5983 2223
Email: mp@asnevents.net.au

Turning Science into Caring

Abbott is a global, broad-based health care company devoted to discovering new medicines, new technologies and new ways to manage health.

Abbott's products span the continuum of care from nutritional products and laboratory devices and pharmaceutical therapies. Abbott's comprehensive line of products encircles life itself addressing important health needs from infancy to the golden years.

Please contact your local representative for more information.

Abbott Products Pty Ltd (Solvay Pharmaceuticals is now Abbott)
Building 2, Level 1, 20 Bridge St
Pymble NSW 2073 Call 1300 300 532

Abbott
A Promise for Life

KEITH HARRISON MEMORIAL LECTURERS

The Keith Harrison Memorial Lecture is given each year at the ESA Annual Scientific Meeting in honour of Prof Keith Harrison, one of the founders of ESA and an early President.

1964	Kenneth Ferguson
1965	Geoffrey Harris
1973	Albert Renold
1974	Paul Franchimont
1975	William Odell
1976	John Landon
1977	Hugh Niall
1978	Samuel Yen
1979	John Shine
1980	Ronald Swerdloff
1981	Sidney Ingbar
1982	Jens Rehfeld
1983	Philip Lowry
1984	Fernand Labrie
1985	Michael Berridge
1986	Michael Thorner
1987	Lynn Loriaux
1988	Axel Ulrich
1989	Hiroo Imura
1990	Iain McIntyre
1991	Eli Adashi
1992	Jan-Ake Gustafsson
1993	Eberhard Nieschlag
1994	Allen Speigel
1995	Natalie Josso
1996	Gregory Mundy
1997	M.Geoffrey Rosenfeld
1998	Ken Korach
1999	Henry Burger
2000	Pierre Chambon
2001	Jack Martin
2002	George Chrousos
2003	Derek LeRoith
2004	Bruce McEwen
2005	Richard Pestell
2006	William Crowley
2007	Gerard Karsenty
2008	Colin Ward
2009	John Cidlowski
2010	Stafford Lightman

NOVARTIS JUNIOR INVESTIGATOR AWARD

The Novartis Junior Award is awarded annually to a member who is a postgraduate student or recent post-doctoral student, for the best original paper at the Annual Scientific Meeting.

1976	Kathryn Rich & Peter Fuller
1977	David Kennaway
1978	David Healy
1979	George Werther
1980	Rebecca Mason
1981	Yvonne Hodgson
1982	David Hurley
1983	Carolyn Scott
1984	David James
1985	Guck Ooi
1986	Marie Ranson
1987	Lora Hutchinson
1988	Vasilious Papadopoulos
1989	David Phillips
1990	Sharon Gargosky
1991	Marie-Christine Keightley & Helen Maclean
1992	Fiona Young
1993	Emma Ball
1994	Vicki Clifton
1995	Michael Downes & Sylvia Lim-Tio
1996	John Walsh
1997	Bu Yeap
1998	Julie Joyner
1999	Renea Jarred & Helena Teede
2000	Jeremy Smith
2001	Stephen Heady
2002	Patrick McManamny
2003	Sophie Chan
2004	Esme Hatchell
2005	Agnes Kovacic & Amy Au
2006	David Macintyre
2007	Marrianne Elston
2008	Sue Lau
2009	Kenneth Ho

ESA BRYAN HUDSON CLINICAL ENDOCRINOLOGY AWARD

The ESA CSL Biotherapies Bryan Hudson Clinical Endocrinology Award will recognize the best clinical research presentation at the Annual Scientific Meeting by an active member of the Endocrine Society of Australia early in their career. It will be made on an annual basis.

2004	Sonia Davison
2005	Carolyn Allan
2006	Jui Ho
2007	Morton Burt
2008	Ann McCormack
2009	Paul Lee

ESA / IPSEN INTERNATIONAL TRAVEL GRANT

This award supports younger members of the society to travel to international meetings, laboratories and/or clinics to further their training and knowledge in Endocrinology.

2003 Emma Ball
2004 Gordon Howarth, Sophie Chan and Vincenzo Russo
2005 Stuart Ellem
2006 Kevin Pfleger and Erosha Premaratne
2007 Lisa-Marie Atkin, Elspeth Gold and Michael Stark
2008 Elif Ekinci, Andrew Siebel, Jenny Chow
2009 Michelle Van Sinderen, Jyotsna Pippal and Ulla Simanainen

SERVIER AWARD

The Servier Award is made annually to recognise the best scientific paper published in the 12-month period preceding the closing date for abstracts for the Annual Scientific Meeting by an active member of the Endocrine Society of Australia early in their career.

1991 Sharon Gargosky
1992 Peter Stanton
1993 Janet Martin
1994 Chen Chen
1995 Timothy Crowe
1996 Jun-Ping Lui
1997 Liza O'Donnell
1998 Stephen Twigg
1999 Dan Lee
2000 Fraser Rogerson
2001 Karen Kroeger
2002 Susan Fanayan
2003 Jenny Gunton
2004 Peter Liu
2005 Simon Chu
2006 Renea Taylor
2007 Kirsten McTavish
2008 Belinda Henry
2009 Kristy Brown

ESA MID-CAREER RESEARCH AWARD

The ESA Mid-Career Researcher Award is designed to recognise an outstanding mid-career researcher in endocrinology.

2009 Rachel Davey

HONORARY LIFE MEMBERS

Dr Robert Baxter
Dr Alan W. Blackshaw
Dr Hal D. Breidahl
Prof James B Brown
Prof Henry G Burger
Dr Robin A. Burston
Prof Donald P Cameron
Prof John P Coghlan
Prof Alex Cohen
Dr Ron I Cox
Prof David Curnow
Dr Ewan Downie
Prof David De Kretser
Prof Creswell J Eastman AM
Cliff W. Emmens
Dr Ken A. Ferguson
Prof John W Funder
Prof Peter Fuller
Prof Richard D. Gordon
Dr Ian B Hales
Prof David Handelsman
Dr Philip Harding
Prof Basil Hetzel
Dr Brian Hirschfeld
Bryan Hudson
Dr Ivan G Jarrett
Assoc Prof Stephen Judd
Prof Richard G Larkins
Prof Leslie Lazarus
Dr Thomas B. Lynch
Prof Ian McDonald
Prof T John Martin
Dr Len Martin
Dr Frank I.R. Martin
Dr Ian C A Martin
Ian R. McDonald
Dr Roger Melick
Prof Solomon Posen
Prof Marilyn Renfree
Prof Gail Risbridger
Prof Terry J. Robinson
Prof Rodney Shearman
Prof Alfred W Steinbeck
Prof Jim Stockigt
Prof Roderick Strang
Prof Pincus Taft
Dr Ian D Thomas
Prof Duncan Topliss
Prof Prof Victor Trikojus
Emeritus Prof John R Turtle
Prof Robert Vines
Dr Alan L. Wallace
Prof Norman Wettenhall
Prof F. H Wilson
Prof Marelyn Wintour-Coghlan
Dr Ken N. Wynne

CONFERENCE SPONSORS

The conference gratefully acknowledges the support of the following organisations:

CONFERENCE PRINCIPAL SPONSORS

Lilly

Johnson & Johnson MEDICAL

MSD

sanofi aventis
Because health matters

CONFERENCE MAJOR SPONSORS

Abbott
A Promise for Life

AMGEN

gsk GlaxoSmithKline

Supported by Amgen in collaboration with GSK

IPSEN
Innovation for patient care

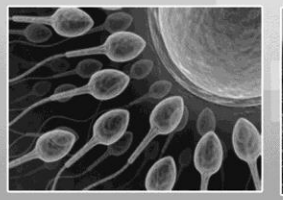
NOVARTIS

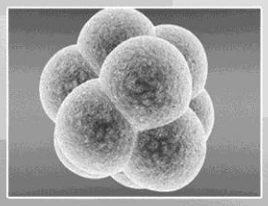
novo nordisk®

The conference acknowledges the sponsorship of sanofi aventis
Because health matters

FoundAnimals *is proud to announce:*

The Michelson Prize & Grants in Reproductive Biology






The Michelson Prize in Reproductive Biology:

A **$25 million Prize** will be awarded to the first entity to provide a non-surgical sterilant that is safe, effective, and practical for use in cats and dogs.

Winning entry must meet the following criteria:

- Single dose, non-surgical sterilant
- Safe and effective in male and female, cats and dogs
- Viable pathway to regulatory approval
- Feasible manufacturing process and cost
- Suitable for administration in a field setting

The Michelson Grants in Reproductive Biology:

Up to **$50 million** in multiple, multi-year grants for promising research pursuing Prize goals.

Grant recipients are eligible for Prize claims.

The first step in the Grant process is submitting a Letter of Intent.

For more information about submitting a Letter of Intent, visit: www.foundanimals.org.

For more information about the Prize and Grants, visit: www.foundanimals.org

Join the mailing list to receive the latest news and updates.

The Alliance for Contraception in Cats and Dogs (ACC&D) is a strategic partner in the Michelson Prize and Grants in Reproductive Biology. Visit www.acc-d.org for information on non-surgical approaches to pet overpopulation.

About Found Animals

Found Animals Foundation, Inc. (Found Animals) is a privately funded 501(c)(3), non-profit organization dedicated to minimizing pet euthanasia in animal shelters. **Found Animals** creates programs designed to reduce shelter intake, increase adoption rates and focuses on developing cost-effective, scalable, and sustainable animal welfare business models.

INVITED PLENARY SPEAKER PROFILES 2010

HARRISON LECTURER

Stafford Lightman - Stafford Lightman is Professor of Medicine at the University of Bristol in the United Kingdom and is Director of the Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology. He started his scientific career working on catecholamines and opioid peptides with Leslie Iversen at the University of Cambridge and provided some of the first data linking opioid peptides with the regulation of neurohypophysial function. At this time he also performed some of the first studies demonstrating the importance of brain stem catecholamine pathways in the regulation of hypothalamic activity. On moving to what is now Imperial College in London, he started to develop his studies on the role of the brain in the regulation of the stress response. He demonstrated the shift from CRH to arginine vasopressin in the control of the hypothalamic-pituitary-adrenal axis during chronic stress, demonstrated and characterised the development of stress hyporesponsiveness during lactation in both rats and man and developed models of immunological activation of the stress response. More recently he has developed the concept of the importance of digital signalling inherent in the pulsatile release of glucocorticoid hormones and has been able to demonstrate the specificity of mineralocorticoid receptor and glucocorticoid receptor responsiveness to rapid changes in levels of circulating glucorticoids.

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

TAFT LECTURER

Karel Pacak - Dr. Pacak together with his colleagues/collaborators introduced a novel biochemical test, the measurement of plasma free metanephrines, in the biochemical diagnosis of pheochromocytoma; introduced a novel clonidine-suppression test; refuted a glucagon test; introduced new reference ranges for plasma free metanephrines for children; introduced a new nuclear imaging method, [18F]-6F-dopamine positron emission tomography (PET) scanning in the diagnostic localization of pheochromocytoma; introduced a “flip-flop” theory in functional imaging of pheochromocytoma using FDG PET scanning; introduced guidelines for functional imaging of these tumors, described mechanisms linking different biochemical and clinical phenotypes and exocytosis in pheochromocytomas in multiple endocrine neoplasia type 2A (MEN 2A) and von Hippel-Lindau (VHL) syndrome; distinct histopathologic phenotypes between MEN 2A and VHL pheochromocytomas; described how NE transporter system can be modulated by HDAC inhibitors (patented) and its possible application as a “sensitizer” before 131I-MIBG treatment; introduced the first successful radiofrequency ablation in the treatment of metastatic pheochromocytoma; described clinical characteristics of SDHB-related pheochromocytomas; introduced guidelines for screening of pediatric patients with SDHB gene mutations, described the role of metastasis suppressor genes in the pathogenesis of malignant pheochromocytoma; proposed the role of IL-13PE in the treatment of these tumors; described a new way using SDHB immunostaining in the diagnosis of SDHx-related pheochromocytomas, found that mediastinal paragangliomas and GIST tumors are related to SDHB gene mutations. Introduced and established International Symposia on Pheochromocytoma, organized patient-oriented conferences: SDHB-Related Pheochromocytoma in 2006, Pheochromocytoma 2007, and 2009 and established Pheochromocytoma Research and Support Organization (PRESSOR). He gave numerous Endocrine Grand Rounds to educate endocrinologists in the area of chromaffin cell tumors.

PLENARY LECTURER

Jayne Franklyn - Qualified MBChB (with Honours) at the University of Birmingham, UK in 1979. Subsequently awarded MD and PhD for research into thyroid hormone action and TSH gene regulation whilst MRC Training Fellow and Wellcome Trust Senior Clinical Fellow. Appointed Professor of Medicine, University of Birmingham and Consultant Endocrinologist, University Hospitals Birmingham, UK in 1995. Longstanding laboratory and clinical research interests focussing on the pathogenesis and long term consequences of thyroid cancer and autoimmune thyroid disease. She is a Fellow of the Academy of Medical Sciences and has been awarded the Royal College of Physicians Goulstonian Lectureship, as well as Plenary Lectureships of the Society for Endocrinology, Clinical Endocrinology Trust, the International

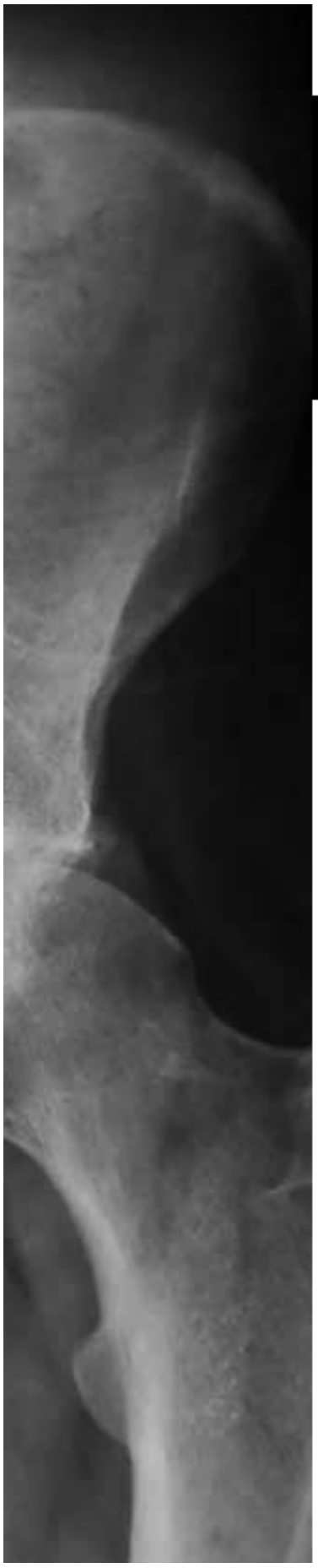
Congress of Endocrinology and both Keynote and Paul Starr Lectureships of the American Thyroid Association. Head of the School of Clinical and Experimental Medicine at the College of Medical and Dental Sciences of the University of Birmingham since 2008 and an active teacher and mentor, as well as researcher, with over 250 peer reviewed papers in thyroid research.

ESA / ADS LECTURER

Ralph DeFronzo - Ralph A. DeFronzo, MD, is Professor of Medicine and Chief of the Diabetes Division at the University of Texas Health Science Center and the Audie L. Murphy Memorial VA Hospital in San Antonio, Texas. Dr. DeFronzo is a graduate of Yale University (BS) and Harvard Medical School (MD) and did his training in Internal Medicine at the Johns Hopkins Hospital. He completed fellowships in endocrinology at the National Institutes of Health and Baltimore City Hospitals and in Nephrology at the Hospital of the University of Pennsylvania. Subsequently, he joined the faculty at the Yale University School of Medicine (1975-88) as an Assistant/Associate Professor.

His major interests focus on the pathogenesis and treatment of type 2 diabetes mellitus and the central role of insulin resistance in the metabolic-cardiovascular cluster of disorders known collectively as the Insulin Resistance Syndrome. Using the euglycemic insulin clamp technique in combination with radioisotope turnover methodology, limb catheterization, indirect calorimetry, and muscle biopsy, he has helped to define the biochemical and molecular disturbances responsible for insulin resistance in type 2 diabetes mellitus.

For his work in this area, Dr. DeFronzo received the prestigious Lilly Award (1987) by the American Diabetes Association (ADA), the Banting Lectureship (1988) by the Canadian Diabetes Association, the Novartis Award (2003) for outstanding clinical investigation world wide and many other national and international awards. He also is the recipient of the ADA's Albert Renold Award (2002) for lifetime commitment to the training of young diabetes investigators. Dr. DeFronzo received the Banting Award from the ADA (2008) and the Claude Bernard Award from the EASD (2008). These represent the highest scientific achievement awards given by the American and European Diabetes Associations, respectively. In 2008 Dr. DeFronzo also received the Italian Diabetes Mentor Prize and the Philip Bondy Lecture at Yale. With more than 500 articles published in peer-reviewed medical journals, Dr. DeFronzo is a distinguished clinician, teacher, and investigator who has been an invited speaker at major national and international conferences on diabetes mellitus.



Company sponsored symposium:

Novel perspectives in osteoporosis

Tuesday 31st August 2010 • 7.15 am to 8.20 am (7.20 am start)
Bayside Room 104, Sydney Convention and Exhibition Centre

Chairperson
Professor Geoff Nicholson PhD (Melb), FRACP, FRCP (Lond)
Department of Clinical and Biomedical Sciences,
Barwon Health, Geelong Hospital, Geelong, Vic

Unmet needs in osteoporosis/burden of illness

Speaker Professor Peter Ebeling MD, FRACP
Professor of Medicine, Head of Endocrinology,
Department of Medicine, Royal Melbourne Hospital/
Western Hospital, University of Melbourne, Vic

Treatment of osteoporosis through the use of Prolia® (denosumab)

Speaker Professor Ian R Reid MD, FRACP
Professor of Medicine and Endocrinology,
Department of Medicine, University of Auckland,
New Zealand

AMGEN®

gsk GlaxoSmithKline

Supported by Amgen in collaboration with GSK

Amgen Australia Pty Ltd, Level 7, 123 Epping Road, North Ryde NSW 2113 ABN 31 051 057 428
www.amgen.com.au • GlaxoSmithKline Australia, Melbourne VIC ABN 47 100 162 481
www.gsk.com.au

DOS100065-AUS 07/2010 • AMS45/7/10
PC1004141

The conference acknowledges the sponsorship of sanofi aventis
Because health matters

INFORMATION FOR DELEGATES & PRESENTERS

Venue
Sydney Convention and Exhibition Centre
Darling Drive
DARLING HARBOUR NSW
Ph: +61 2 9282 5000

Registration
The Full Delegate Registration fee includes:
* all delegate materials (name tag, satchel, abstract book)
* lunches (Monday, Tuesday and Wednesday)
* morning teas (Monday, Tuesday and Wednesday)
* afternoon teas (Monday and Tuesday)
* the welcome function

The Day Registration fee includes:
* all delegate materials (name tag, satchel, abstract book)
* lunch for the specified day
* morning tea for the specified day
* afternoon tea for the specified day

Venue Layout
The registration desk is located on the Bayside Ground Level Foyer, which is outside the trade area. All breaks are taken in this space. The plenary lectures and concurrent sessions are mainly held on level one of the convention centre. Please see venue map on the next page.

Organiser's Office and Registration Desk
The organiser's office and registration desk will be located on the Bayside Ground Level Foyer. The registration desk will be open on Sunday 29th August from 12:00 Noon to 6:00 PM and on Monday 30th and Tuesday 31st August from 7:00 AM – 5:30 PM.

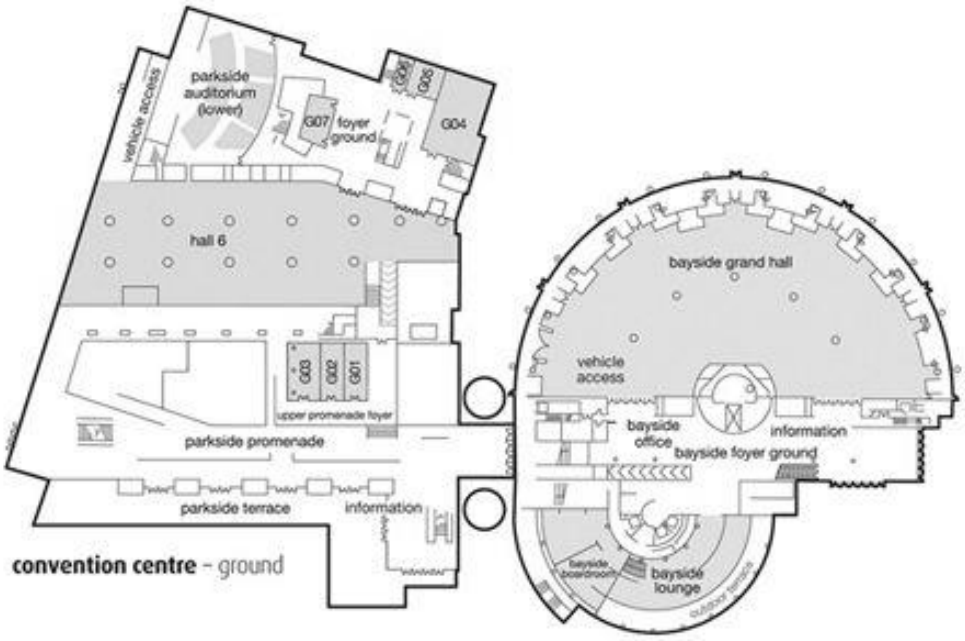
The Speaker Preparation Room
Presentations are to be loaded direct to the PC in the speaker preparation room (Bayside 108) at least a full session in advance of your session. You should bring your talk on a USB, saved in a format for display on a pc within the room. A technician will be on hand to assist with any transfer / loading issues and to help you check your presentation. There are both PCs and Macintosh computers in the speaker preparation room but please note there are no Macintosh computers in the presentation rooms.

Name Tags
Delegates are required to wear their name tags to all scientific and catered sessions. Uniformed security is in attendance on the doors of the exhibition area and name tags are required to gain access.
Delegates should note that within their name tag pouch are any specific function tickets they have ordered as well as a second smaller name tag which should be placed in the holder on your satchel.

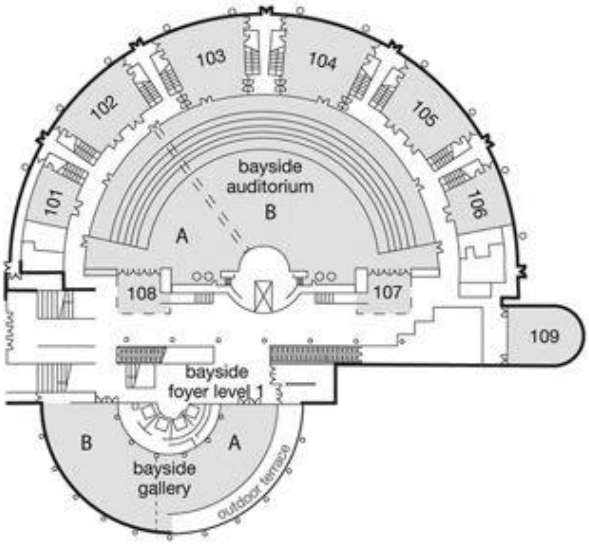
Poster Viewing
Delegates with posters can find the correct position for their poster by finding the appropriate abstract number on the display panels. The panels are set up in the Bayside Gallery. The program provides your abstract number which is how you find you placement position. Posters can remain on display from Monday morning and must be removed by afternoon tea on Tuesday. During formal poster discussions (on Monday afternoon), the presenters should attend their poster to answer questions and meet colleagues with similar research interests. The posters are grouped in categories and refreshments will be served.

Internet Café *The conference acknowledges the sponsorship of Medtronic*
There will be an internet café provided for delegates for the duration of the meeting. The café is located on the left hand side of the exhibition hall in Booth 16B.

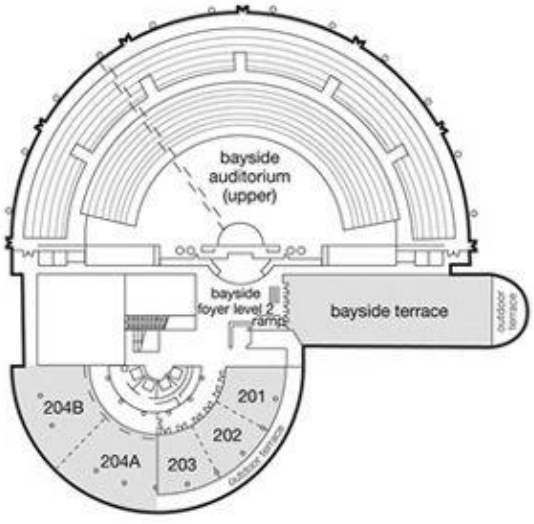
Ground Level



Level One



Level Two



Social Functions

- The **Welcome Function** is at the Sydney Convention and Exhibition Centre on the Sunday evening from 6:00pm. Light refreshments and drinks will be served and the function is complimentary for all registration types. The function will take place in the Bayside Terrace. Additional tickets for partners can be purchased from the registration desk.
- The Monday night **Student to Scholar Social** is being held on a Harbour Cruise. Delegates who have already purchased a ticket should find their ticket with their registration papers on arrival. The ticket cost includes your meal, entertainment and limited drinks. The cruise embarks at 7:00pm from Star City Casino Wharf, Pirrama Road and disembarks at 10:30pm at Star City Casino Wharf, Pirrama Road ('C" on map). There will be an optional disembarkment for those that do not want to attend the entire cruise at 9:00pm at Pier One, Ive Steps. For those that would like to continue the night, delegates are invited to head to the Pyrmont Bridge Hotel. Please head to the general public bar upstairs at the venue. The Pyrmont Bridge Hotel is located at: 96 Union Street, Pyrmont NSW 2009, (02) 9660 6996. Please see map below for directions and points of embarkment and disembarkment and for the location of the Pyrmont Bridge Hotel. Dress is neat casual. **This is a ticketed function** and they must be purchased in advance from the registration desk.
- The **Conference Dinner** will be held on Tuesday evening at the Sydney Convention and Exhibition Centre in the Bayside Gallery. Pre-dinner drinks will be served from 7:00pm for a 7:30pm start. Dress is neat casual. **This is a ticketed function** and they must be purchased in advance from the registration desk.

Insurance - The hosts and organisers are not responsible for personal accidents, any travel costs, or the loss of private property and will not be liable for any claims. Delegates requiring insurance should make their own arrangements.

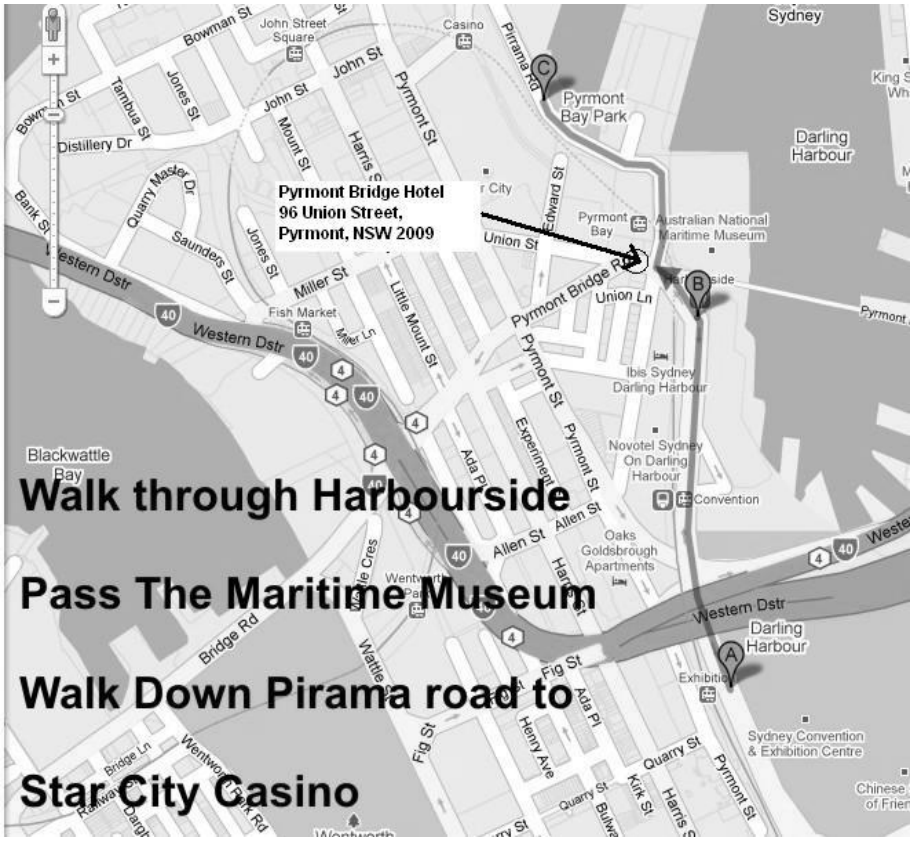
Smoking - is not permitted in the venue.

Mobile Phones - Please ensure they are turned off during any session you attend.

Message Board - will be available at the registration desk.

Occasional Meetings - A number of special meetings and functions have been called by various interested parties throughout the conference. Those involved and uncertain of which room they should be in will be able to obtain guidance from the registration desk.

Disclaimer - The hosts, organisers and participating societies are not responsible for, or represented by, the opinions expressed by participants in either the sessions or their written abstracts.



We take diabetes personally.

Lilly Diabetes

Each person living with diabetes faces individual challenges that require individual solutions. We not only understand that, we're doing something about it. We're committed to providing healthcare professionals and their patients the treatments, tools, education, and support they need to make the journey a successful one. One person at a time.
Your journey inspires ours.

Eli Lilly Australia Pty Ltd ABN 39 000 233 992. 112 Wharf Road, West Ryde 2114. AUDBT00101 ELD3336

Lilly
Answers That Matter.

TRADE BREAKFAST WORKSHOPS

Please visit the registration desk if you would like to attend a trade breakfast workshop and have not let the organisers know already.

Monday 30th August, 2010

Roche
7:15 AM – 8:20 AM Bayside 103

The Value of Blood Glucose Monitoring in Insulin-Naïve Type 2 Diabetes: Results from the Structured Testing Program (STeP) Study

Speaker: William Polonsky
Chair: Reich Webber-Montenegro

While self-monitoring of blood glucose (SMBG) is known to be beneficial among insulin users, its value and utility in insulin-naïve type 2 diabetes (T2DM) remains uncertain. To examine this issue further, we recently completed a 1-year, prospective, cluster-randomized, multi-center, clinical trial to assess the effectiveness of structured SMBG in poorly-controlled (HbA1C ≥7.5%), insulin-naive T2DM subjects. This presentation will describe the study design as well as the interim results, focusing on changes in subjects' glycaemic control, self-care behaviours and quality of life as well as changes in physicians' practice patterns. Recommendations for using structured SMBG in clinical practice will be described.

Tuesday 31st August, 2010

Amgen
7:15 AM – 8:20 AM Bayside 204

Novel Perspectives in Osteoporosis

Chairperson: Professor Geoff Nicholson
Professor Peter Ebeling - Unmet needs in osteoporosis / burden of illness
Professor Ian Reid - Treatment of osteoporosis through the use of Prolia® (denosumab)

Sunday, 29 August 2010

SRB Council Meeting
12:00 PM - 3:00 PM — Bayside 107

Afternoon Tea
3:00 PM - 3:30 PM — Bayside 103

Joint Association for Applied Animal Andrology/SRB Symposium - Andrology in the Future
3:30 PM - 5:30 PM — Bayside 103

The conference acknowledges the sponsorship of

Chairs: David Mortimer (4A) & Gareth Evans (SRB)

3:30pm **R. John Aitken**
Origins of DNA damage in spermatozoa *abs#001*

4:10pm **Bart Gadella**
Pig sperm egg interaction and formation of a zona pellucida binding complex *abs#002*

4:50pm **Stuart Meyers**
Oxidative stress, osmotic stress and apoptotic changes: Effects on equine spermatozoa *abs#003*

ESA Council Meeting
3:30 PM - 5:30 PM — Bayside 107

SRB RCRH Award Winner
5:30 PM - 6:00 PM — Bayside 103

The conference acknowledges the sponsorship of

Chairs: Jeremy Thompson (RCRH) & Kate Loveland (SRB)

Evdokia Dimitriadis
A new era in contraceptive development: non-hormonal options that also target sexually transmitted infections *abs#004*

ESA / SRB Welcome Function
6:00 PM - 7:30 PM — Bayside Terrace

The conference acknowledges the sponsorship of

Women in Endocrinology Function
7:00 PM - 8:00 PM — Bayside 103

The conference acknowledges the sponsorship of

Roche Diagnostics Breakfast Workshop - The Value of Blood Glucose Monitoring in Insulin-Naïve Type 2 Diabetes: Results from the Structured Testing Program (STeP) Study – Professor William Polonsky

7:15 AM - 8:20 AM Bayside 103

ESA Taft Plenary Lecture

8:30 AM - 9:30 AM Auditorium A

Chair: Leon Bach

Karel Pacak

Pheochromocytoma and Paraganglioma: New discoveries to guide clinical practice and future research *abs#005*

SRB Orals One - Spermatogenesis

8:30 AM - 10:00 AM Bayside 105

Chairs: Brett Nixon & Jennifer Ly Huynh

8:30am **Jenna Haverfield**
The relationship between Sertoli cell status and idiopathic male infertility *abs#101*

8:45am **Andrew Reid**
Characterisation of the GTPase dynamin throughout murine sperm maturation *abs#102*

9:00am **Megan Mitchell**
The effect of paternal obesity in mice on reproductive and metabolic fitness of F1 male offspring *abs#103*

9:15am **Kathleen Deboer**
Lrguk – a novel gene involved in male fertility *abs#104*

9:30am **Frank Grutzner**
Meiotic acrobats: monotreme sex chromosome organisation during spermatogenesis *abs#105*

9:45am **Shaun Roman**
Nucleosome retention during chromatin packaging in human spermatozoa *abs#106*

SRB Orals Two - Assisted Reproductive Technologies and Stem Cells

8:30 AM - 10:00 AM Bayside 102

Chairs: David Gardner & Kylie Dunning

8:30am **Chris O'Neill**
Responses of the preimplantation embryo to defined genotoxic stresses *abs#107*

8:45am **Justin St. John**
Interspecies somatic cell nuclear transfer is dependent on compatible cytoplasmic factors and mitochondrial DNA *abs#108*

9:00am **Fleur Oback**
Aggregating cloned with *in vitro* fertilised embryos results in chimaeras and improved fetal suvival in cattle *abs#109*

9:15am **Zamira Gibb**
Dimethyl formamide improves the DNA integrity and motility of sex-sorted cryopreserved stallion spermatozoa *abs#110*

9:30am **Margot Day**
The role of L-proline in preimplantation mouse embryo development *in vitro* *abs#111*

9:45am **Christine Yeo**
Fetal Calf Serum affects hESC metabolism and gene expression leading to differentiation in culture *abs#112*

ESA Servier Award

9:30 AM - 9:45 AM — Auditorium A

Chair: David Phillips

Zoë Hyde
Low Free Testosterone Predicts Frailty In Older Men *abs#201*

ESA Morning Tea

9:45 AM - 10:15 AM — Bayside Grand Hall

SRB Morning Tea

10:00 AM - 10:30 AM — Bayside Grand Hall

ESA Novartis Junior Scientist Award

10:15 AM - 12:00 Noon — Auditorium A

Chairs: Mark McLean and Kenneth Ho

10:15am **Heather Lee**
Progesterone and Elf5 in mammary gland development *abs#202*

10:30am **Shirin Hussain**
Estrogen receptor ß activates TNFα-mediated apoptosis in benign prostatic hyperplasia *abs#203*

10:45am **Jennifer Lo**
The role of RABL2A in protein trafficking and its relation with male fertility and ciliopathies *abs#204*

11:00am **Nicholas Kasmeridis**
Immunisation with oxidised LDL protects against insulin resistance in dietary-induced obesity *abs#205*

11:15am **Christian Girgis**
Atypical Femur Fractures and Bisphosphonate Use *abs#206*

11:30am **Tammy Pang**
Investigating non-genomic signalling pathways of the androgen receptor *abs#207*

11:45am **Lyndal Tacon**
The Glucocorticoid Receptor is Overexpressed and Transcriptionally Active in Malignant Adrenocortical Tumours *abs#208*

SRB Orals Three - ANZPRA Emerging Investigator

10:30 AM - 12:00 Noon — Bayside 105

Chairs: Claire Roberts (ANZPRA) & Jemma Evans (SRB)

10:30am **Prabha Andraweera**
Vascular endothelial growth factor gene polymorphisms in placental impairment and small for gestational age birth *abs#113*

10:45am **Amanda Highet**
Hypertensive disorders of pregnancy are associated with immunoregulatory gene polymorphisms *abs#114*

11:00am **Huiting Ho**
Development of potent and stable PC6 inhibitors to block embryo implantation for female contraception and prevention of HIV *abs#115*

11:15am **Chez Viall**
Trophoblast antiphospholipid antibody internalisation by a $ß_2$ glycoprotein I-anionic phospholipid-megalin complex *abs#116*

11:30am **Yui Kaneko**
The role of focal adhesion proteins and their hormonal regulation in rat uterine epithelial cells during early pregnancy *abs#117*

11:45am **Kirsty Pringle**
The prorenin receptor/PLZF pathway in human amnion *abs#118*

SRB Orals Four - Fertilisation

10:30 AM - 12:00 Noon Bayside 102

Chairs: Chris O'Neill & Phoebe Jennings

10:30am **Kate Redgrove**
Identification and Characterisation of Surface Protein Complexes in Human Spermatozoa *abs#119*

10:45am **Larry Chamley**
SPRASA a potential contraceptive vaccine target? *abs#120*

11:00am **Alexander Sobinoff**
Short term xenobiotic exposure compromises long term oocyte viability *abs#121*

11:15am **Kiri Beilby**
Production of embryos in superovulated ewes using frozen-thawed, sex-sorted and refrozen-thawed sperm *abs#122*

11:30am **Hassan Bakos**
The Effect of Paternal Diet Induced Obesity on Sperm Capacitation, Acrosome Reaction, Binding and Fertilisation in Mouse Model *abs#123*

11:45am **Sarah Dalati**
The role of calcium activated chloride channels at fertilisation *abs#124*

ESA Lunch

12:00 Noon - 1:00 PM Bayside Grand Hall

SRB Founders Lecture

12:00 Noon - 1:00 PM Bayside 103

The conference acknowledges the sponsorship of

Reproduction, Fertility and Development

Chair: Mark Hedger

Renee Reijo Pera
Human Germ Cell Formation and Differentiation from Pluripotent Stem Cells *abs#006*

ESA Meet the Professor: Research Leadership

12:10 PM - 1:00 PM Bayside 104

Chair: Helena Teede

John Shine
Research Leadership *abs#007*

SRB Lunch

1:00 PM - 2:00 PM Bayside Grand Hall

SRB Annual General Meeting

1:00 PM - 2:00 PM Bayside 102

ESA Orals - Bone and Calcium (Clinical)

1:00 PM - 2:30 PM Auditorium A

The conference acknowledges the sponsorship of

Supported by Amgen in collaboration with GSK

Chairs: Peter Ebeling and Jackie Center

1:00pm **Thuy Vu**
'Capturing' Fractured Patients: improving management of osteoporosis in a tertiary hospital *abs#209*

1:15pm **Jackie Center**
Osteoporosis Medication And Mortality Risk In Elderly Women And Men: An 18-year Prospective Study from Dubbo Osteoporosis Epidemiology Study *abs#210*

1:30pm **Richard Prince**
The effects of a two year RCT of whey protein supplementation on bone density and urinary calcium excretion in older postmenopausal women *abs#211*

1:45pm **Peter Ebeling**
Denosumab (DMAb) Effects on Bone Mineral Density (BMD) and Fracture Stratified by Baseline Level of Renal Function *abs#212*

2:00pm **Thuy Vu**
Increased fracture risk in patients with newly diagnosed type 1 diabetes mellitus maybe partly due to reduced cortical density *abs#213*

2:15pm **Joshua Lewis**
Calcium supplementation and the risk of atherosclerotic vascular disease in postmenopausal women *abs#214*

ESA Orals - Cancer (Basic)

1:00 PM - 2:30 PM Bayside 105

Chairs: Mathis Grossman and Maree Bilandzic

1:00pm **Kara Britt**
Is the parity induced decreased breast cancer risk due to a decrease in estrogen sensitivity in the breast? *abs#215*

1:15pm **Kyren Lazarus**
Function of LRH-1 in Breast Cancer *abs#216*

1:30pm **Roxanne Toivanen**
Stromal Androgen Receptor-Mediated Paracrine Signaling Enhances the Efficiency of Xenografting Human Localised Prostate Cancer *abs#217*

1:45pm **Gail Risbridger**
Targeting castrate resistant prostate cancer tumours with estrogen therapy *abs#218*

2:00pm **Maree Bilandzic**
Invasive behaviours in human granulosa cell tumours are associated with loss of betaglycan and the downregulation of *MMP-15* and *MMP-16* mRNAs *abs#219*

2:15pm **Stacey Jamieson**
The FOXL2 C134W mutation is pathognomonic for adult granulosa cell tumours of the ovary *abs#220*

ESA Orals - Diet, Obesity and Metabolism (Basic)

1:00 PM - 2:30 PM Bayside 104

Chair: Iain Clarke

1:00pm **Karen Chiam**
In Utero Exposure to a High Fat Diet is Associated with an Increased Incidence of Prostate Abnormalities and Mirna Expression Changes in Adult Rat Offspring *abs#221*

1:15pm **Dana Briggs**
Diet-Induced Obesity Alters the Central Actions of Ghrelin *abs#222*

1:30pm **Hayden John Leonard McEwen**
Suppressor of Cytokine Signalling 3 (SOCS3) is Partially Responsible for High Fat Diet-Induced Infertility Through Suppression of the Preovulatory LH Surge *abs#223*

1:45pm **Isabelle Lys**
Glucocorticoid regulation of lipolysis genes and the role of mineralocorticoid receptors during adipogenesis *abs#224*

2:00pm **Belinda Henry**
Alpha-melanocyte stimulating hormone (αMSH) acts directly on muscle to increase putative thermogenesis *abs#225*

2:15pm **Sue Lynn Lau**
Loss of 1,25$(OH)_2$Vitamin-D3 in beta-cells causes impaired glucose tolerance and glucose-stimulated insulin secretion: metabolic effects of 1-α-hydroxylase (Cyp27B1) knockout in insulin-producing cells *abs#226*

ESA/SRB Joint Symposium - Pregnancy Nutrition (Basic)

2:00 PM - 4:00 PM Bayside 103

The conference acknowledges the sponsorship of

sanofi aventis
Because health matters

Chairs: Larry Chamley (SRB) & Beverly Muhlhausler (ESA)

2:00pm **Maria Makrides**
N-3 long chain polyunsaturated fatty acids: the good oils for pregnancy outcome? *abs#008*

2:30pm **Suyinn Chong**
Maternal ethanol consumption alters the epigenotype and phenotype of offspring in a mouse model *abs#009*

3:00pm **Frank Bloomfield**
Periconceptional undernutrition: life-long effects for the offspring *abs#010*

3:30pm **Janet Rowan**
Gestational diabetes – complications, management, outcomes *abs#011*

SRB Orals Five - Ovary & Folliculogenesis

2:00 PM - 4:00 PM Bayside 102

Chairs: Ray Rogers & Michael Bertoldo

2:00pm **Charles Allan**
Elevated FSH increases primordial follicle reserve without increasing primordial follicle formation or decreasing oocyte quality *abs#125*

2:15pm **Jessie Sutherland**
JAK/STAT Signalling in Folliculogenesis *abs#126*

2:30pm **F. Hamish Morgan**
Puma mediates germ cell death during ovarian development and determines initial primordial follicle number in mice *abs#127*

2:45pm **Courtney Simpson**
Structural analysis of GDF-9 mutations associated with premature ovarian failure and twinning *abs#128*

3:00pm **Ann Drummond**
Ovarian phenotype of the IKK conditional knockout mouse *abs#129*

3:15pm **Laura Watson**
Conditional targeted deletions of STAT3 to identify its role in oocytes and granulosa cells *abs#130*

3:30pm **Leanne Pacella**
SIRT3 in ovarian cells is altered by maternal age and ovarian reserve *abs#131*

3:45pm **Davina Cossigny**
Activin A has a stimulatory effect *in vitro* on early follicle development in rat ovaries *abs#132*

ESA/Neuroendocrinology Australasia Joint Symposium - Generation and Significance of Pulsatile Hormone Secretion (Basic)

2:30 PM - 4:30 PM Bayside 104

The conference acknowledges the sponsorship of

Chairs: Brian Oldfield and Belinda Henry

2:30pm **Stafford Lightman**
Does the Pattern of Glucocorticoid Secretion Matter? *abs#012*

3:00pm **Paul Le Tissier**
Microcirculation and endocrine systems: Imaging pituitary function at cellular resolution *in vivo* *abs#013*

3:30pm **Iain Clarke**
Pulsatile GnRH secretion *abs#014*

4:00pm **Penny Hawken**
The smell of sex – pheromones and GnRH secretion *abs#015*

ESA Symposium - Thyroid Function and Disease (Clinical)

2:30 PM - 4:30 PM Auditorium A

Chairs: Duncan Topliss and Jeremy Hoang

2:30pm **Jayne Franklyn**
Management and long term consequences of subclinical thyroid disease *abs#016*

3:00pm **John Walsh**
What Is Normal Thyroid Function, Anyway? *abs#017*

3:30pm **Creswell Eastman**
Iodine deficiency in Australia and overseas *abs#018*

4:00pm **Vijay Panicker**
Genetic variation and thyroid hormone function *abs#019*

ESA / SRB Poster Viewing Session

4:00 PM - 6:00 PM Bayside Gallery

See Poster Listing

SRB Election of Early Career Researcher Representative

6:00 PM - 7:00 PM Bayside 201

SRB Students Annual Meeting

6:00 PM - 7:00 PM Bayside 102

Genzyme Thyroid Workshop
6:00 PM - 8:00 PM

Bayside 103

Student to Scholar Social - Harbour Cruise
7:00 PM - 10:30 PM

Embark - Star City Casino Wharf, Pirrama Road

The conference acknowledges the sponsorship of

NOVARTIS

Amgen - GSK Breakfast Workshop - Novel Perspectives in Osteoporosis

7:20 AM - 8:20 AM Bayside 104

ESA / SRB Poster Viewing

8:00 AM – 2:00 PM Bayside Gallery

ESA Plenary Lecture

8:30 AM - 9:30 AM Auditorium A

Chair: Warrick Inder

Jayne Franklyn

Pathogenesis of thyroid diseases *abs#020*

R F & D Award Plenary Lecture

8:30 AM - 9:30 AM Bayside 103

The conference acknowledges the sponsorship of

Reproduction, Fertility and Development

Chairs: Tony Flint (Editor in Chief RF&D) & Mark Hedger (SRB)

David Handelsman

Hormonal Regulation of Spermatogenesis *abs#021*

ESA / SRB Morning Tea

9:30 AM - 10:00 AM Bayside Grand Hall

ESA/SRB Joint Orals - Female Reproduction (Basic)

10:00 AM - 12:00 AM Auditorium A

Chair: Darryl Russell (SRB) and Jeremy Smith (ESA)

10:00am **Mohammad Johan**
Lesion weight and glandular development are suppressed in a TGFB1 deficient mouse model of endometriosis *abs#133*

10:15am **Jeremy Smith**
Kisspeptin is essential for the full preovulatory LH surge in sheep *abs#227*

10:30am **Louie Ye**
Differentiation of human embryonic stem cells to Mullerian tissue *abs#134*

10:45am **Nicolette Hodyl**
Sex-specific regulation of glucocorticoid exposure in preterm pregnancies by P-gp and MRP-1 *abs#228*

11:00am **Grant Montgomery**
Genome-wide association study identifies a locus at 7p15.2 associated with the development of moderate-severe endometriosis *abs#135*

11:15am **Hannah Palliser**
IUGR induced upregulation of PGHS-1 contributes to increased risk of preterm birth *abs#229*

11:30am **Lisa Akison**
Progesterone receptor-regulated genes in the preovulatory ovarian follicle and oviduct *abs#136*

11:45am **Annette Osei-Kumah**
Expression of human leukocyte antigen by monocyte subsets during pregnancy *abs#230*

SRB Orals Six - Gene Regulation in Reproduction

10:00 AM - 12:00 Noon Bayside 103

Chairs: Eileen McLaughlin & Keri Beilby

10:00am **Jessica Stringer**
Genomic Imprinting in the Marsupial Mammary Gland *abs#137*

10:15am **Jodie Painter**
Genome-wide linkage scan for familial dizygotic twinning *abs#138*

10:30am **Patricia Grant**
Placental expression of microRNAs alters with gestation in the guinea pig *abs#139*

10:45am **Keng Yih Chew**
Genes controlling phallus development *abs#140*

11:00am **Eugenie Lumbers**
Methylation of genes of the renin angiotensin system (RAS) in early human amnion *abs#141*

11:15am **Skye McIver**
MicroRNA and early male germ cells *abs#142*

11:30am **Helen Irving-Rodgers**
Differences in gene expression between apical and basal cells of the membrana granulosa *abs#143*

11:45am **Shu Ly Lim**
Maelstrom- a protein that is essential for spermatogenesis and transposable repression is expressed in adult ovary of mammals and birds *abs#144*

ESA Symposium - Recent Advances in Cancer Therapies and Mechanisms (Basic)

10:00 AM - 12:00 Noon Bayside 204

Chairs: Lisa Butler and Reidun Aesoey

10:00am **Carmela Ricciardelli**
Targeting the cancer-peritoneal interaction in ovarian cancer *abs#022*

10:30am **Bruce Robinson**
Endocrine Tumour Genetics - Advances in Diagnosis and Treatment *abs#023*

11:00am **Nigel McMillan**
Towards RNAi therapy for Cancer – Solving the Critical Issues *abs#024*

11:30am **Colleen Nelson**
Insulin increases de novo steroidogenesis in castrate resistant prostate cancer- potential for new therapeutic approaches *abs#025*

ESA Symposium - Endocrinology and Energy Balance (Basic/Clinical)

10:00 AM - 12:00 Noon Bayside 104

Chairs: Greg Cooney and George Muscat

10:00am **Peter Clifton**
Endocrine and metabolic consequences of weight loss and of different weight loss approaches *abs#026*

10:30am **Brian Oldfield**
New Frontiers in Energy Expenditure – the Role of Brown Adipose Tissue *abs#027*

11:00am **Jeffrey Zajac**
The metabolic effects of androgens on energy balance and voluntary activity *abs#028*

11:30am **Ian Caterson**
Weight, Weight Loss & Insulin Resistance *abs#029*

SRB Lunch

12:00 Noon - 1:00 PM Bayside Grand Hall

The conference acknowledges the sponsorship of

AMGEN gsk GlaxoSmithKline

Supported by Amgen in collaboration with GSK

ESA Harrison Plenary Lecture

12:00 Noon - 1:00 PM Auditorium A

Chair: Tim Cole

Stafford Lightman
Why does activity of the HPA axis oscillate? The importance of continuous dynamic equilibration *abs#030*

SRB Meet the Professor – Students

12:00 Noon - 1:00 PM Bayside 103

Moderator: Charles Allan

David Handelsman
Terry Hassold
Pat Hunt
Eva Dimitriadis

SRB Meet the Professor - Early Career Researchers

12:10 PM - 1:00 PM Bayside 104

Moderator: Eileen McLaughlin

Keith Jones
John Aitken
Moira O'Bryan
Renee Reijo Pera

ESA Lunch

1:00 PM - 2:00 PM Bayside Grand Hall

The conference acknowledges the sponsorship of

AMGEN gsk GlaxoSmithKline

Supported by Amgen in collaboration with GSK

SRB New Investigator Award

1:00 PM - 3:00 PM Bayside 204

The conference acknowledges the sponsorship of

MERCK SERONO

Chair: Mark Hedger

1:00pm **Stefan Sonderegger**
Wingless (Wnt)3A induces trophoblast migration and Matrix metalloproteinase-2 secretion through canonical Wnt signalling and protein kinase B/AKT activation *abs#145*

1:15pm **Kylie Dunning**
Molecular filtration properties of the expanded cumulus matrix: Controlled supply of metabolites and extracellular signals to cumulus cells and the oocyte *abs#146*

1:30pm **Francine Marques**
Molecular characterization of renin-angiotensin system components in human intrauterine tissues and fetal membranes from vaginal delivery and caesarean section *abs#147*

1:45pm **Peter Nicholls**
Hormonally regulated miRNAs target the tubulobulbar complex in the testis *abs#148*

2:00pm **Kara Gunter**
Translational Control in Folliculogenesis and Oocyte Development: A role for RNA-binding protein Musashi-1 *abs#149*

2:15pm **Elise Pelzer**
In vitro characterisation of biofilm formation in human follicular fluid *abs#150*

ESA Meet the Expert

1:15 PM - 2:00 PM Bayside 104

Chair: Grant Montgomery

Valerie Wasinger
Peptide enrichment and quantitative profiling to facilitate marker discovery in plasma *abs#031*

NZSE Annual General Meeting

1:30 PM - 2:00 PM Bayside 103

ESA Orals - Bryan Hudson Clinical Award and Case Studies

2:00 PM - 4:00 PM Auditorium A

Chairs: Bu Yeap and Jui Ho

Bryan Hudson Clinical Award

2:00pm **Jeremy Hoang**
Evaluation of Neuroendocrine Tumours using Somatostatin Receptor PET *abs#231*

2:15pm **Zoe Hyde**
Testosterone levels predict sexual activity in older men *abs#232*

2:30pm **Paul Lee**
The metabolic effect of a highly β2-selective agonist, formoterol, in humans *abs#233*

Case Studies

2:45pm **Shantha Joseph**
Phaeochromocytoma, back with a bite - many faces, many dilemmas *abs#234*

3:00pm **Nirjhar Nandi**
Acromegaly – Where is the tumour? *abs#235*

3:15pm **Mark Pace**
PET SCANcer *abs#236*

3:30pm **Georgina Thomas**
Not Enough Fat: Lipodystrophy & Leptin Deficiency *abs#237*

3:45pm **Jarod Sze Choong Wong**
Intra-uterine growth retardation (IUGR) due to 11p15 loss of methylation: Endocrine and metabolic consequences *abs#238*

ESA Orals - Early Life Programming and IUGR (Basic)

2:00 PM - 4:00 PM Bayside 104

Chairs: Brendan Waddell and Nicolette Hodyl

2:00pm **Tamara Varcoe**
Exposure of rats to a simulated shift work schedule during pregnancy impacts the health and development of the offspring *abs#239*

2:15pm **Mary Wlodek**
Calcium Supplementation does not Rescue the Programmed Adult Bone Deficits Associated with Perinatal Growth Restriction *abs#240*

2:30pm **Himawan Harryanto**
Placental Restriction Alters MicroRNAs Expression in Skeletal Muscle, Liver and Adipose Tissue Young and Adult Offspring in the Sheep *abs#241*

2:45pm **Ezani Mohamed Jamil**
Maternal folic acid supplementation and abundance and expression of insulin-like growth factor-II in offspring *abs#242*

3:00pm **Mary Wlodek**
Being Born Small Programs Nephron Deficits and Hypertension in the Next Generation *abs#243*

3:15pm **Hong Liu**
Periconceptional undernutrition alters fetal pancreatic ß-cell mass in late gestation *abs#244*

3:30pm **Siti Sulaiman**
Neonatal Exendin-4 Treatment Increases Beta-Cell Mass and Alters Islet Gene Expression in the IUGR Lamb *abs#245*

3:45pm **Melanie Tran**
Being Born Small Programs Fetal Growth Restriction and Pancreatic Deficits in the Subsequent Generation *abs#246*

SRB Afternoon Tea

3:00 PM - 3:30 PM Bayside Grand Hall

SRB Orals Seven - Implantation & Pregnancy

3:30 PM - 5:30 PM Bayside 103

Chairs: Wendy Ingman & Natalie Hannan

3:30pm **Guiying Nie**
Placental HtrA3 is regulated by oxygen tension and serum levels are altered during early pregnancy in women destined to develop preeclampsia *abs#151*

3:45pm **Ellen Menkhorst**
Development of a vaginally applied, non-hormonal contraceptive: the contraceptive efficacy and impact on bone turnover of PEGLA, a long-acting LIF antagonist *abs#152*

4:00pm **Stephen Tong**
Combination Methotrexate and Epidermal Growth Factor Receptor Inhibition as a novel medication-based cure of ectopic pregnancies *abs#153*

4:15pm **Neil Gude**
Extracellular calreticulin alters endothelial cell and trophoblast cell functions *in vitro* consistent with pre-eclampsia *abs#154*

4:30pm **Natalie Hannan**
Understanding the crosstalk in the human uterine cavity: roles for soluble mediators during embryo implantation *abs#155*

4:45pm **Sophea Heng**
Identification of scaffolding proteins as PC6 substrates in the human endometrial epithelial cells for embryo implantation *abs#156*

5:00pm **Peter Rogers**
Lymphatics in the Human Placental Bed and Surrounding the Spiral Arterioles Disappear During Endometrial Decidualisation *abs#157*

5:15pm **Peter Mark**
The unfolded protein response may contribute to glucocorticoid-induced placental growth restriction in the rat via increased placental expression of heat shock protein 70 *abs#158*

SRB Symposium - Meiosis and Beyond

3:30 PM - 5:30 PM Bayside 204

The conference acknowledges the sponsorship of

CBD
ARC Centre of Excellence in Biotechnology & Development

Chairs: Frank Grutzner & Duangporn Jamsai

3:30pm **Terry Hassold**
The origin of aneuploidy in humans: where we've been, where we're going *abs#032*

4:10pm **Keith Jones**
Cdh1: a cell cycle protein involved in female meiosis and prevention of aneuploidy *abs#033*

4:50pm **Jennifer Ly-Huynh**
Importin α2 mediates subnuclear targeting of the Cajal body component Coilin; a key role in spermatogenesis? *abs#034*

ESA Afternoon Tea

4:00 PM - 4:30 PM Bayside Grand Hall

ESA Mid-Career Award Lecture

4:30 PM - 5:00 PM Auditorium A

Chair: Vicki Clifton

Peter Liu
Age-related changes in the regulation of sleep and the male gonadal axis *abs#247*

ESA Annual General Meeting

5:00 PM - 6:00 PM Auditorium A

ESA / SRB Conference Dinner

7:00 PM - 11:00 PM Bayside Gallery

The conference acknowledges the sponsorship of

novo nordisk®

ESA Breakfast Career Workshop for Junior Clinicians

7:00 AM - 8:25 AM — Bayside 204

Chair: John Walsh

7:00am **Emma Duncan**
Balancing all your commitments in a clinical research career

7:30am **Leon Bach**
Career pathways in clinical research *abs#036*

8:00am **Jui Ho**
Getting your first paper published *abs#037*

ESA Breakfast Career Workshop for Junior Scientists

7:00 AM - 8:25 AM — Bayside 201 and 202

Chair: Miles De Blasio

7:00am **Moira O'Bryan**
Establishing an independent research career *abs#038*

7:30am **Gail Risbridger**
Making a Competitive CV *abs#039*

7:45am **Mike Waters**
Attributes of a successful postdoc

SRB Orals Eight - Female Reproductive Tract

8:30 AM - 10:30 AM — Bayside 103

Chairs: Viv Perry & Ellen Menkhorst

8:30am **Jemma Evans**
Extracellular matrix dynamics in scar-free endometrial repair *abs#159*

8:45am **Qi Chen**
The role of phagocytosis of apoptotic syncytial knots in the prevention of endothelial cell activation: an important adaptation for normal pregnancy *abs#160*

9:00am **Claire Roberts**
Predicting gestational hypertension and preeclampsia from maternal angiotensin II and angiotensin 1-7 levels at 15 weeks gestation *abs#161*

9:15am **Jane Fenelon**
Friend or foe? The roles of paf and p53 during embryonic diapause *abs#162*

9:30am **Caroline Gargett**
Potential Markers for the Prospective Isolation of Human Endometrial Epithelial Stem/Progenitor Cells *abs#163*

9:45am **Sarah Paule**
Integrin cleavage is mediated by proprotein convertase 6 in human endometrial epithelial cells for endometrial receptivity and implantation *abs#164*

10:00am **Wendy Ingman**
Location of active TGFB1 in the mammary gland during different stages of development *abs#165*

10:15am **Ying Li**
Embryo implantation is associated with specific expression of proprotein convertase 6 in the rabbit uterus *abs#166*

SRB Symposium - Environment and Fertility

8:30 AM - 10:30 AM — Bayside 201 and 202

The conference acknowledges the sponsorship of

CBD
ARC Centre of Excellence in Biotechnology & Development

Chairs: Kate Loveland & Shaun Roman

8:30am **Patricia Hunt**
Are environmental exposures affecting human reproductive health? *abs#041*

9:10am **Richard Lim**
Endocrine disrupting impacts in receiving waters of the Sydney Basin *abs#042*

9:40am **Stuart Linton**
The effect of the insecticide pyriproxyfen on ovary synthesis in the Christmas Island red crab, *Gecarcoidea natalis*; a possible case of endocrine disruption? *abs#043*

ESA/ADS Plenary Lecture

8:30 AM - 9:30 AM — Auditorium A

Chairs: Kathy Gatford and Jenny Gunton

Ralph DeFronzo
Prevention and Treatment of Type 2 Diabetes: A Sound Approach Based Upon Its Pathophysiology *abs#044*

Joint ESA/AWE Symposium - AWE Showcase (Basic/Clinical)

9:30 AM - 11:00 AM — Bayside 204

Chair: Gail Risbridger

9:30am **Gail Risbdridger**
Welcome

9:40am **Carolyn Allan**
Testosterone, Body Composition and the Ageing Male *abs#045*

10:00am **Sue Mei Lau**
Mechanisms of obesity in the offspring of diabetic pregnancy *abs#046*

10:20am **Theresa Hickey**
Androgen receptor signalling in human breast tissues and its implications for breast cancer *abs#047*

10:40am **Sarah To**
The role of TNFα in oestrogen biosynthesis and breast cancer *abs#048*

ESA Symposium - Endocrine Receptors and Signal Transduction (Basic)

9:30 AM - 11:00 AM — Bayside 104

Chairs: Christine Clarke and Vita Birzniece

9:30am **Prof. Peter Leedman**
RNA-binding cofactors in nuclear receptor-mediated gene regulation *abs#049*

10:00am **Mike Waters**
Novel Mechanisms in GH signaling *abs#050*

10:30am **George Muscat**
The orphan nuclear hormone receptor NR4A subgroup, targets of beta-adrenergic signaling: Understanding the role of NR4A3/NOR-1 in in skeletal muscle metabolism *abs#051*

ESA/ADS Symposium - Choosing and using Thresholds in Diabetes Diagnosis and Management (Clinical)

9:30 AM - 11:00 AM Auditorium A

The conference acknowledges the sponsorship of

MSD

Chairs: Morton Burt and Michael d'Emden

9:30am **Peter Colman**
HbA1c – Things are changing - Diagnostic role and new reporting *abs#052*

10:00am **Aidan McElduff**
Choosing and using Thresholds in Diabetes Diagnosis and Management: Gestational Diabetes *abs#053*

10:30am **Pat Phillips**
Other targets in diabetes *abs#054*

SRB Morning Tea

10:30 AM - 11:00 AM Bayside Grand Hall

ESA Morning Tea

11:00 AM - 11:30 AM Bayside Grand Hall

ESA/SRB Joint Orals - Male reproduction: from testis to brain

11:00 AM - 1:00 PM Bayside 103

Chair: Charles Allan (SRB) and Catherine Choong (ESA)

11:00am **Eileen McLaughlin**
Musashi family of RNA binding proteins: cell cycle regulators in spermatogenesis *abs#167*

11:15am **Amanda Idan**
Clinical Pharmacology of Human Chorionic Gonadotrophin: Randomized Sequence Cross-over Study of Recombinant vs Urinary hCG in Gonadotrophin Suppressed Healthy Men *abs#248*

11:30am **Mai Sarraj**
TGFß 2-betaglycan regulate foetal testis development *in vitro* *abs#168*

11:45am **Ulla Simanainen**
Proof that Human AR Q-tract Length Determines Androgen Sensitivity *In Vivo* *abs#249*

12:00pm **Duangporn Jamsai**
The Mechanism of Spermatid Maturation – A Link to Tumour Suppression *abs#169*

12:15pm **Mathis Grossmann**
Androgen Deprivation Therapy for Prostate Cancer Increases Visceral Fat Mass and Insulin Resistance *abs#250*

12:30am **Melissa Gamat**
The role of megalin in prostate development of the mouse *abs#170*

12:45pm **Camilla Hoyos**
Longer-term effects of testosterone therapy on sleep, breathing and body composition in obese men with Obstructive Sleep Apnea (OSA) undergoing weight loss: A randomised placebo controlled 18 week trial *abs#251*

ANZPRA/SRB Symposium - Placentae through the Millennia

11:30 AM - 1:00 PM Bayside 201 and 202

Chairs: Peter Rogers (SRB) & Peter Mark (ANZPRA)

11:30am **Marilyn Renfree**
Trophoblast, placenta and early embryo: how the marsupial develops *abs#055*

12:10pm **Michael Thompson**
Even reptiles do it, the structure and function of placentae in viviparous lizards *abs#056*

12:50pm **Brendan Waddell**
Eutherian mammals do it differently: placental endocrine strategies for the maintenance of pregnancy in rodents and primates *abs#057*

ESA Orals - Clinical Management and Screening (Clinical)

11:30 AM - 1:30 PM Bayside 102

Chair: Michael Stowasser

11:30am **Bronwyn Stuckey**
Low urinary iodine postpartum is associated with hypothyroid postpartum thyroid dysfunction and predicts long term hypothyroidism *abs#252*

11:45am **Paul Fogarty**
Mandatory Iodine Fortification of Bread in Australia: Iodine Status of Pregnant Women *abs#253*

12:00pm **Ashraf Ahmed**
Is the phase of the menstrual cycle important when screening for primary aldosteronism in women, and does renin assay method matter? *abs#254*

12:15pm **Morton Burt**
Assessment of the Hyperglycaemic Effect of Prednisolone During an Exacerbation of Chronic Obstructive Pulmonary Disease with a Continuous Glucose Monitoring System *abs#255*

12:30pm **Anju Joham**
Pigment epithelium derived factor as a marker of cardiometabolic risk factors and insulin resistance in overweight women with and without polycystic ovary syndrome *abs#256*

12:45pm **Paul Lee**
Brown adipose tissue is present in the majority of adult humans: histologic and molecular characterization of PET positive and negative supraclavicular fat *abs#257*

1:00pm **Eduardo Pimenta**
Unilateral Adrenalectomy Improves Urinary Protein Excretion But Does Not Abolish Its Relatioship to Sodium Excretion in Patients Wit Aldosterone-Producing Adenoma *abs#258*

1:15pm **Ashraf Ahmed**
Effect of atenolol on aldosterone/renin ratio calculated by both plasma renin activity and direct renin concentration in healthy male volunteers *abs#259*

ESA Orals - Receptors and Signaling (Basic)

11:30 AM - 1:30 PM Bayside 105

Chairs: Ken Ho and Helen Atkinson

11:30am **Christine Clarke**
Overlapping functional effects of progesterone in mouse and human mammary gland have distinct transcriptional signatures *abs#260*

11:45am **Yan Ru Gao**
Androgen receptor DNA binding is not required for normal mammary gland structure and function in mice *abs#261*

12:00pm **Jorge Tolosa**
A new function for the fusogenic endogenous retroviral envelope protein Syncytin-1: Assessment of its immunosuppressive properties *abs#262*

12:15pm **Yi Chen**
Tumour necrosis factor-α stimulates human neutrophils to release pre-formed activin A *abs#263*

12:30pm **Sarah To**
Early Growth Response transcription factors stimulate CYP19A1 expression in response to TNFα – novel mechanisms of oestrogen biosynthesis *abs#264*

12:45pm **Sze Yee Chai**
Epigenetic regulation of progesterone receptor isoform expression in term human myometrium *abs#265*

1:00pm **Helen Atkinson**
Chronic ICV infusion of oCRF eliminates the corticosterone diurnal rhythm while maintaining pulsatility *abs#266*

1:15pm **Vita Birzniece**
Interaction between testosterone and growth hormone on whole body protein anabolism is mediated through the liver *abs#267*

SRB Lunch
1:00 PM - 2:00 PM Bayside Grand Hall

ESA Lunch
1:30 PM - 3:00 PM Bayside Grand Hall

ESA Meet the Professor: Research in Type II Diabetes
2:00 PM - 3:00 PM Bayside 204

The conference acknowledges the sponsorship of

Johnson & Johnson MEDICAL

Ralph DeFronzo
Insulin Resistance, Type 2 Diabetes, and ASCVD: The Missing Links *abs#058*

SRB / ADS Plenary Lecture
2:00 PM - 3:00 PM Auditorium B
Chairs: Chris Nolan and Janet Rowan

N Wah Cheung
Gestational Diabetes and Type 2 Diabetes in Pregnancy in Australia *abs#059*

ESA Expert Opinions: Calcium Safety and Calcium/Vitamin D efficacy in Osteoporosis
3:00 PM - 5:00 PM Bayside 204
Chair and Moderator: David Torpy

3:00pm **David Torpy**
Introduction

3:05pm **Richard Prince**
Calcium and vitamin D are first line effective osteoporotic fracture prevention interventions *abs#060*

3:25pm **Graeme Jones**
Calcium and Vitamin D efficacy: what are the facts? *abs#061*

3:45pm **Ian Reid**
Safety of Calcium and Vitamin D in Osteoporosis *abs#062*

4:050pm **Chris Nordin**
Safety of calcium supplements in postmenopausal women *abs#063*

SRB Orals Nine - Oocytes and Embryos

3:00 PM - 5:00 PM Bayside 102

Chairs: Chris Grupen & Xingliang Jin

3:00pm **Natalie Binder**
Parental obesity retards early embryonic development and alters carbohydrate utilisation *abs#171*

3:15pm **Peck Chin**
A modest inflammatory insult in the pre-implantation period alters oviduct cytokine expression and programs fetal development *abs#172*

3:30pm **Lakshi Ganeshan**
Trp53 -dependent expression of genes regulating embryo viability *abs#173*

3:45pm **Petra Wale**
Atmospheric oxygen alters the embryonic metabolome as quantified by carbohydrate uptake and amino acid utilisation *abs#174*

4:00pm **Jia Yi (Joy) Lin**
Effects of species differences on oocyte regulation of granulosa cell proliferation *abs#175*

4:15pm **Michael Bertoldo**
Inhibition of porcine oocyte nuclear maturation *in vitro* using a phosphodiesterase inhibitor and an adenylate cyclase activator *abs#176*

SRB Orals Ten - Testis and Sperm Function

3:00 PM - 5:00 PM Bayside 105

Chairs: Graeme Martin & Kathleen DeBoer

3:00pm **Ilona Ciller**
Antibodies against BMPR-IB Uncovers a Paracrine Function for the Receptor in Male Mouse Leydig Cell Testosterone Production *in vitro* *abs#177*

3:15pm **Matthew Dun**
The Chaperonin Containing TCP-1 (CCT/TRiC) Multisubunit Complex is Involved in Mediating Sperm-Oocyte Interactions *abs#178*

3:30pm **Wendy Winnall**
Purification and characterisation of mouse testicular macrophages: gene expression response to lipopolysaccharide activation indicates an immunosuppressive phenotype *abs#179*

3:45pm **Belinda Jean Nixon**
The Consequences of Acrylamide Exposure on the Male Germline *abs#180*

4:00pm **Nicole Palmer**
SIRT6 protein is reduced in testes and sperm from obese male mice *abs#181*

4:15pm **Adam Koppers**
Role of CRISP4 in ion channel regulation and male reproduction *abs#182*

PRESCRIBE JANUVIA + METFORMIN FOR TYPE II DIABETES PATIENTS PREVIOUSLY UNCONTROLLED ON METFORMIN ALONE.

Please review the Product Information before prescribing. Product Information is available from MSD.

MINIMUM PRODUCT INFORMATION: Indications: Type 2 Diabetes Mellitus, in persons ≥ 18 years old, who have failed dietary measures and exercise as dual combination therapy with metformin or with a sulphonylurea, or with a thiazolidinedione where the use of a thiazolidinedione is considered appropriate. **Contraindications:** Hypersensitivity to any component of the product. **Precautions:** Renal insufficiency; hypoglycaemia in combination with a sulphonylurea; pregnancy (Cat B3); lactation; children; not for Type 1 Diabetes Mellitus or diabetic ketoacidosis. **Interactions:** No clinically significant interactions observed with metformin, glibenclamide, simvastatin, rosiglitazone, warfarin, oral contraceptives, digoxin or cyclosporin. (see full PI). **Adverse reactions:** URTI, nasopharyngitis, headache, Hypoglycaemia when used in combination with a sulphonylurea. **Postmarketing experience:** Hypersensitivity reactions, Pancreatitis, Worsening renal function (including acute failure).* **Dosage:** 100 mg once-daily. Adjust for moderate and severe renal insufficiency (see full PI). Consider reduced dose of sulphonylurea when used in combination. Tablet Rx (S4) sitagliptin; lactose free; 25 mg (pink), 50 mg (light beige), 100 mg (beige). Pack size; [28], [7] -Starter Pack. **JANUVIA Tablets:** PBS dispensed price $91.19. **References: 1.** JANUVIA Approved Product Information. **2.** Nauck M, Meninger G, Sheng D et al. Diabetes Obes Metab 2007; 9:194–205. **3.** Data on file. MSD Australia.

JAN-10-AUS-6143-J. First issued June 2010. 8747_A4.

*Please note changes in Product Information.

MSD

PBS Information: Authority Required (STREAMLINED). Refer to PBS Schedule for full authority information.

ESA POSTER LISTING

Monday 30th August, 4:00 PM – 6:00 PM

Cancer

Reidun Aesoey
Mechanisms of ERβ Action in Prostate Cancer *abs#401*
Bree Cawsey
Defining the role of estrogen and mast cells in prostatitis and the development and progression of prostate cancer *abs#402*
Simon Chu
Expression and cellular activation of peroxisome proliferator-activated receptor gamma in granulosa cell tumours *abs#403*
Kevin Knower
Novel effects of melatonin on oestrogen production in post-menopausal breast cancer *abs#404*
Sarah Wilkinson
Stromal Hedgehog Signaling Mediates Prostate Epithelial Transformation *abs#405*

Case Studies

Wilton Braund
Entrapping a Body-builder. A Case Report *abs#406*
Ada Cheung
Tumour shrinkage and improved headache with octreotide in a TSH and GH co-secreting pituitary macroadenoma *abs#407*
Emily Gianatti
The Enigma of Ectopic ACTH Syndrome: Case Report and Update *abs#408*
Christian Girgis
Multifocal Fibrosclerosis in a Patient with a Progressive Goitre and Hypothyroidism *abs#409*
Kiernan Hughes
Bonsoy and Iodine: Too much of a good thing *abs#410*
Anju Joham
An Intriguing Case of Cushing's *abs#411*
I-Lynn Lee
Thunderclap in the toilet *abs#412*
Nirjhar Nandi
A case of Sarcoidosis with hypercalcemia and renal involvement in an Indigenous lady *abs#413*
Rashmi Narayanan
Aypical presentations of pituitary apoplexy *abs#414*
Rashmi Narayanan
Revisiting the diagnosis of phaeochromocytomas *abs#415*
Mark Ng Tang Fui
Another reason to avoid the dentist: metastatic insulinoma *abs#416*
Shalini Nilajgi
To present a rare case of pseudo hypoparathyroidism presenting with polyglandular failure *abs#417*
Mark Pace
Therapeutic Options in Treatment-Refractory Acromegaly *abs#418*
David Pattison
Exercise Associated Hyponatraemia: an Australasian Case Series *abs#419*
Nimalie Perera
The Complexity of Laboratory Testing and Diagnosis of Steroid Excess Syndromes associated with Herbal Remedy Use *abs#420*

Annalise Pham
Myositis associated with treated Hyperthyroidism *abs#421*
Agata Piotrowicz
Investigations and management challenges of dedifferentiation follicular thyroid cancer *abs#422*
Walter Plehwe
Acute Adrenal Infarction: How often is it overlooked? *abs#423*
Praseetha Shanmugalingam
CASE REPORT: Isolated ACTH deficiency presenting as severe hypercalcaemia with acute renal failure *abs#424*
Emershia Suthaharan
An interesting case of Lymphocytic hypophysitis and thyroiditis *abs#425*
Jenny Tu
An Atypical Case of Sitagliptin (Januvia) and Atorvastatin Related Proximal Myopathy *abs#426*
Tang Wong
High grade IGF-II producing sarcoma causing recurrent hypoglycemia in a recently gravid 29 year old woman *abs#427*

Clinical Studies

Carmela Caputo
Gender differences in hormonal outcome following surgery for non-functioning pituitary adenomas *abs#428*
Santosh Chaubey
Evaluation of 25 (OH) vitamin D status in chronic kidney disease patients of north Queensland *abs#429*
David Hoffman
Efficacy of Glargine Insulin in Type 2 Diabetes - An Audit of Clinical Practice Using Audit4 *abs#430*
Ashley Makepeace
Assessment of Pre- and Post-Fortification Iodine Status in the West Australian Population *abs#431*
James McFarlane
Localisation, transiency and independence of hair cortisol responses to a brief pain stressor *abs#432*
Mark Ng Tang Fui
Low testosterone levels in Type 1 compared to Type 2 diabetic men *abs#433*
Parul Nigam
Hyperthyroxinemia with non suppressed TSH –A diagnostic dilemma *abs#434*
Parul nigam
Von Hippel-Lindau Disease presenting as recurrent and multiple phaeochromocytoma *abs#435*
Eduardo Pimenta
Dietary Sodium Increases the Severity of Obstructive Sleep Apnea in Patients with Aldosterone Excess and Resistant Hypertension *abs#436*
Eduardo Pimenta
Cardiac Structure is Largely Influenced by Dietary Salt in Patients with Primary Aldosteronism but not in Patients with Normal Aldosterone *abs#437*

Clinical Studies - Bone and Calcium

Abhay Daniel
A study of Osteoporosis in patients admitted to hospital with low trauma fracture *abs#438*
Jennifer Ng
Is total calcium adequate for the diagnosis of PTH-dependent hypercalcaemia? *abs#439*
Thuy Vu
Parathyroid Hormone Excess and Deficiency: is Peter really robbed or Paul paid? *abs#440*
Ming li Yee
Bone Mineral Density and Body Composition in women with Turner Syndrome *abs#441*

Eduardo Pimenta

Correction of Aldosterone Excess by Adrenalectomy Reduces Salt Appetite in Patients with Aldosterone-Producing Adenoma *abs#442*

Khoa Truong

Graves' disease and Vitamin D deficiency in Vietnamese living in Melbourne *abs#443*

Hui Wu

Serum activin A as an independent predictor of type 2 diabetes mellitus *abs#444*

Bu Yeap

Serum total osteocalcin level predicts mortality in older men. The Health In Men Study. *abs#445*

Bu Yeap

Divergent associations of insulin-like growth factor binding proteins 1 and 3 with mortality in older men. The Health In Men Study *abs#446*

Neuroendocrinology

Xiaobing Cheng

Investigating androgen receptor-mediated androgen action in the neuroendocrine regulation of ovulation *abs#447*

Qun Li

Kisspeptin Cells in the Ovine Arcute Nucleus Express Prolactin Receptor (Long-Form) but not Melatonin Receptor (MT1) *abs#448*

Farid Moslemipur

Effect of Orexin on LH and FSH Secretions in Pre-and Post-Pubertal Rams Fed Restricted Diet *abs#449*

Stephanie Simonds

Elevated leptin levels in obesity increase blood pressure by sympatho-excitation. *abs#451*

Farid Moslemipur

Intravenous Injections of Neuropeptide Y Caused A Hyperthyroidism in Male Goats *abs#450*

Obesity and Metabolism

Shaffinaz Abd Rahman

Systems Biology Approach to Identify Novel Biomarkers of Metabolic Disease in Obese Children Utilizing Nuclear Hormone Receptor Gene Expression Profiling *abs#452*

Vita Birzniece

Validation of Multi-Frequency Bioelectrical Impedance Analysis (MFBIA) for measurements of body composition: a study of growth hormone and testosterone administration in healthy adults *abs#453*

Chloe Cheung

Implication of genetic variants near *FTO*, *GNPDA2* and *MC4R* with persistent central obesity and the metabolic syndrome in Southern Chinese *abs#454*

Marianne Diaz

c-Ski Transgenic Mice Are Partially Resistant to Diet-Induced Obesity *abs#455*

Pablo Enriori

A parallel circuit in glucose homeostasis: α-Melanocortin Stimulating Hormone from pituitary enhance glucose uptake by skeletal muscle *abs#456*

Mehmet Kemal Erbil

Plasma levels of the atherogenic molecules, soluble CD40 Ligand, p-selectin and von-Willebrand factor in subjects with impaired glucose tolerance *abs#457*

Natalie Eriksson

Candidate based expression profiling of a muscle specific Nr4a3 transgenic mouse model *abs#458*

Warrick Inder

Skeletal muscle 11ßHSD1 activity is not influenced by central obesity or insulin resistance in non-diabetic subjects *abs#459*

Farid Moslemipur

Streptozotocin-Induced Hypoinsulinemia Causes Endocrine Changes in Male Sheep *abs#460*

Beverly Cheok Kuan Ng

Effects of High Fat Diet Stress On Vitamin D Receptor Deficient Mice *abs#461*

Michael Pearen

The nuclear hormone receptor, Nor-1 (NR4A3), a target of ß-adrenoreceptor signalling, controls insulin sensitivity and energy homeostasis in skeletal muscle *abs#462*

Ovulation, Pregnancy and Parturition

Prabha Andraweera

Hypoxia-Inducible Factor-1α gene polymorphisms and the risk of preeclampsia in a Sinhalese population from Sri-Lanka *abs#463*

Eng-Cheng Chan

Differential expression of microRNA with labour in the human myometrium *abs#464*

Nicolette Hodyl

Maternal plasma circulating levels of omega (n)3 long chain polyunsaturated fatty acids, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) are associated with fetal growth measures *abs#465*

Kathryn Gatford

Circulating growth hormone profiles remain pulsatile during pregnancy in pigs *abs#466*

Jessica Lewis

The inflammatory state of the rat placenta increases at term and is further elevated by exposure to dexamethasone *abs#467*

Kaushik Maiti

GPR30, the novel membrane estrogen receptor, stimulates contractility of myometrium by increasing expression of h-caldesmon *abs#468*

Carolyn Mitchell

CpG island methylation of proinflammatory and steroid receptor gene promoters in the human amnion *abs#469*

Greg Ong

Is seasonal variation in Vitamin D deficiency during pregnancy reflected by seasonal variation in preeclampsia and gestational diabetes mellitus? *abs#470*

Jonathan Paul

Caldesmon Phosphorylation and Phasic regulation of ERK 1/2 during contractions in Human Myometrium *abs#471*

Stacey Plug

The effect of anthelmintics on ovarian function in Merino ewes *abs#472*

Toni Welsh

Decreased uterine progesterone receptor expression may explain functional progesterone withdrawal in the pregnant guinea pig *abs#473*

Prematurity and Programming

Wing Hong Vincent Chu

Differential expression of hepatic microRNAs in prenatally folic acid supplemented neonates *abs#474*

Rebecca Dyson

Preterm birth and intrauterine growth restriction: effect on microvascular function in the neonatal guinea pig *abs#475*

Himawan Harryanto

IUGR Alters rno-miR-16, -18a and -142-3p in Day 260 Omental Fat BUVL Rat Offspring Tissue *abs#476*

Meredith Kelleher

Premature birth results in *ex utero* brain development in a low neuroprotective steroid environment *abs#477*

Saidatul Mohammad

Maternal folic acid supplementation in the rat alters pancreatic gene expression in adult progeny. *abs#478*

Mary Wlodek

The Impact of Pregnancy and Lactation on Bone in Rat Mothers Exposed to Uteroplacental Insufficiency *abs#479*

Steroid Hormones

Lisa Butler

Differential effects of exogenous androgens and inhibition of androgen signalling in the developing and mature murine mammary gland *abs#480*

Ilona Ciller

BMPR-IB has a Developmental Role in Testosterone Production in Male Mice. *abs#481*

Nicole KL Lee

Odc1 and *Tceal7* are potential mediators of androgen actions in skeletal muscle *abs#482*

Keely McNamara

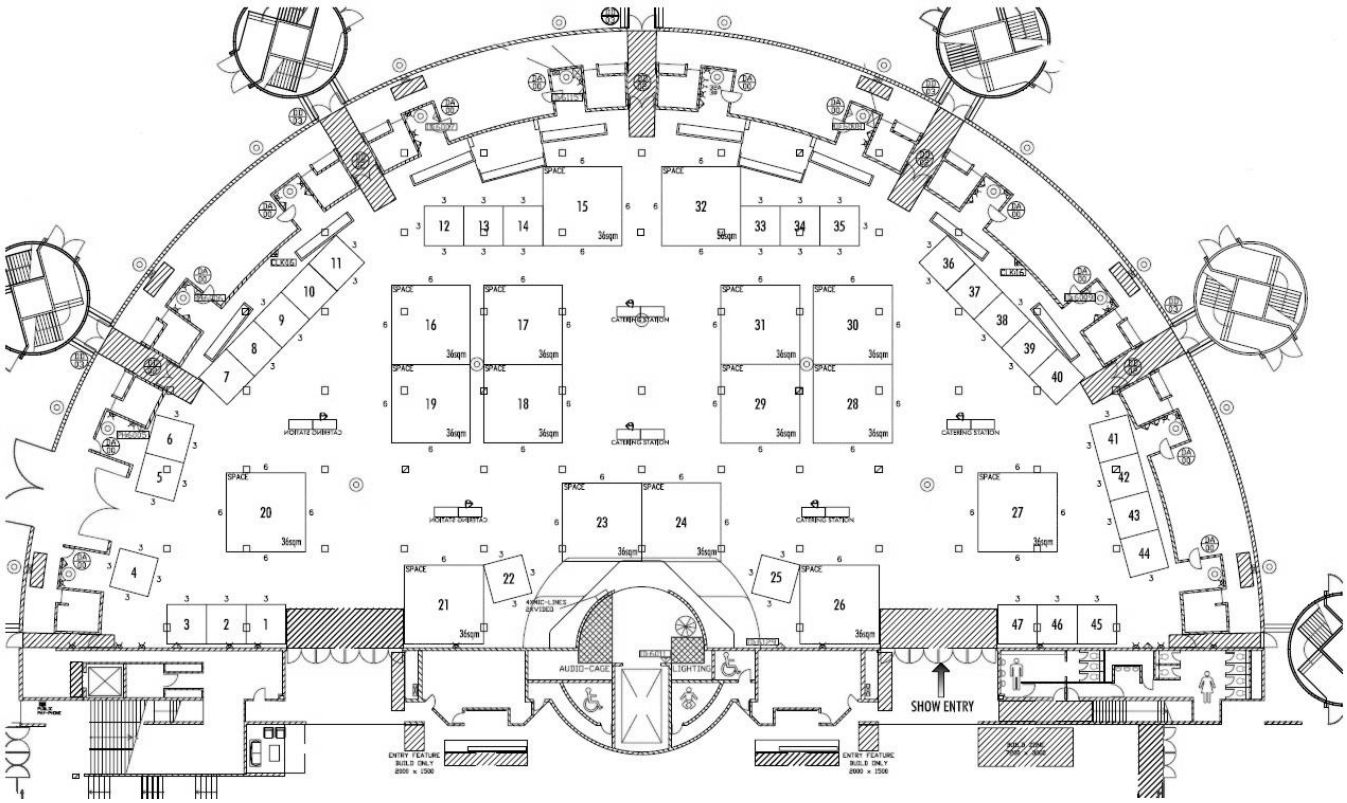
Androgen regulation of 5α-Reductase type 2 in the mouse prostate. *abs#483*

Kirsty Walters

Androgen receptor-mediated actions play a role in regulating late follicular dynamics and embryo development past the 2-cell stage *abs#484*

Yao Wang

TGF-betas and activin regulation of steroidogenesis in TM3 Leydig cells is SMAD2-dependent *abs#485*



Abacus ALS **Booth 47**
12 Mowbray Terrace
East Brisbane QLD 4169
Ph: 1800 222 287, Fax: 1800 287 222
Contact: Pauline Hawkins
Email: p.hawkins@abacus-als.com
Web: www.abacus-als.com
Abacus ALS supplies and supports a wide range of quality products for both clinical and research applications, including an automated system for Bone, Growth and Cartilage assays (IDS-iSYS). Visit our exhibition to receive your special delegate 20% discount voucher on hormone related products.

Abbott Diabetes Care **Major Sponsor, Booth 19**
666 Doncaster Road
Doncaster VIC 3109
Ph: 03 9843 7130, Fax: 03 9855 8020
Contact: Kelly Beck
Email: kelly.beck@abbott.com
Web: www.abbottdiabetescare.com.au
Abbott Diabetes Care helps people with diabetes live healthy and active lives through innovative glucose monitoring technology, support and education.
Since 1991 Abbott Diabetes Care has brought to Australia leading glucose monitoring products such as the Optium Xceed and FreeStyle Lite to help people living with diabetes.
Diabetes is one of Australia's national health priorities. Abbott Diabetes Care undertakes R&D to continually evolve and improve glucose monitoring to help people manage their diabetes and provide flexible lifestyle choices.

Abbott Products (formely Solvay) **Booth 23**
Building 2, Level 1, 20 Bridge Street
Pymble NSW 2073
Ph: 02 9440 0977, Fax: 02 9440 0910
Contact: Genevieve Coburn
Email: Genevieve.Coburn@solvay.com

Abbott is a global, broad-based health care company devoted to the discovery, development, manufacture and marketing of pharmaceuticals and medical products, including nutritionals, devices and diagnostics. The company employs approximately 83,000 people and markets its products in more than 130 countries.

Alphapharm **Booth 1**
Cnr Wentworth Park Road and Bay Street
Glebe NSW 2036
Ph: 0417 248 231, Fax: 02 9298 3992
Contact: Justin Lawrence
Email: justin.lawrence@alphapharm.com.au
Web: www.alphapharm.com.au
Alphapharm has been researching, developing and making medicines in Australia for more than 25 years and we've become the largest supplier of medicines to the subsidised PBS. Through our Cardio-metabolic franchise, we have been leaders in the Diabetic market since 1997 and continue to focus our efforts on improved dosing and delivery options for Diabetic patients.

Amgen - GSK **Major Sponsor**
Level 7, Avaya House, 123 Epping Rd
North Ryde NSW 2113
Ph: 02 9170 1369, Fax: 02 9518 3066
Contact: Kate McKay
Email: esa.amgen@podium.com.au
Web: www.amgen.com.au
Amgen discovers, develops, manufactures and delivers innovative human therapeutics. A biotechnology pioneer since 1980, Amgen was one of the first companies to realize the new science's promise by bringing safe and effective medicines from lab, to manufacturing plant, to patient. Amgen therapeutics have changed the practice of medicine, helping millions of people around the world in the fight against cancer, kidney disease, rheumatoid arthritis, bone loss, and other serious illnesses. With a deep and broad pipeline of potential new medicines, Amgen remains committed to advancing science to dramatically improve people's lives. To learn more about our pioneering science and our vital medicines, visit www.amgen.com
GlaxoSmithKline – one of the world's leading research-based pharmaceutical and healthcare companies – is committed to improving the quality of human life by enabling people to do more, feel better and live longer. For further information please visit www.gsk.com.

Bayer Australia Limited **Booth 39**
875 Pacific Highway
Pymble NSW 2073
Ph: 02 9391 6176, Fax: 02 9391 6633
Contact: Melinda Bacon
Email: laura.duchini@bayerhealthcare.com
Web: www.bayerscheringpharma.de
Bayer is an international, research-based enterprise with core competencies in three business areas: health care, nutrition and high-tech materials. These key activities are represented by three business groups: Bayer HealthCare, Bayer CropScience and Bayer MaterialScience.
Bayer has had a presence in Australia since 1925 with approximately 850 employees.

Beckman Coulter **Booth 38**
Unit D, 24 College Street
Gladesville NSW 2111
Ph: 1800 060 880, Fax: 02 9844 6096
Contact: Moira Rooney
Email: australianz_sales@beckman.com
Web: www.beckmancoulter.com
Beckman Coulter provides solutions for scientists involved in complex biomedical testing. We offer large range of quality products in RIA and ELISA formats, covering assays in endocrinology, oncology, and other fields of diagnostics. Our range of reproductive assays include AMH, Inhibin A, Inhibin B, PAPP-A and ultrasensitive estradiol. For more information, visit www.beckmancoulter.com or contact australianz_sales@beckman.com.

Boehringer Ingelheim **Booth 30**
78 Waterloo Road
North Ryde NSW 2113
Ph: 02 8875 8700, Fax: 02 8875 8712
Contact: Malcolm Riddell

Email: malcolm.riddell@boehringer-ingelheim.com
Web: www.boehringer-ingelheim.com
Boehringer Ingelheim is an independent family-owned company dedicated to researching, developing, manufacturing and marketing innovative products of high therapeutic value for human and veterinary medicine. It is one of the world's 20 leading pharmaceutical companies.
In **Australia and New Zealand**, Boehringer Ingelheim employs around 350 staff who work across our four main business functions:

- Prescription Medicine
- Consumer Health Care
- Animal Health
- Duboisia Farms

Bristol-Myers Squibb – AstraZeneca **Booth 24**
556 Princes Hwy
Noble Park North VIC 3174
Ph: 03 9213 4000, Fax: 03 9701 1526
Contact: Matthew Baker
Email: matthew.baker@bms.com
Web: www..bmsa.com.au
Bristol-Myers Squibb and AstraZeneca entered into a collaboration in January 2007 to enable the companies to research, develop and commercialize two investigational drugs for type 2 diabetes. The Bristol-Myers Squibb/AstraZeneca Diabetes collaboration is dedicated to global patient care, improving patient outcomes and creating a new vision for the treatment of type 2 diabetes.

Eli Lilly **Principal Sponsor, Booth 29**
112 Wharf Road
West Ryde NSW 2114
Ph: 02 9325 4444, Fax: 02 9325 4410
Contact: Benita Saad
Email: SAAD_BENITA_JOANNE@LILLY.COM
Web: www.lilly.com
With its 90 year history of developing diabetes treatments, Lilly is proud to offer health care providers and patients a wide range of therapies and devices for both type 1 and type 2 diabetes.
Lilly continually invests in diabetes research studies both in Australia and globally, and supports educational programs such as Best Practice in Diabetes Centres in collaboration with the NADC.
Lilly proudly shares a passion and commitment to continue to improve the lives of millions of people in Australia and around the world affected by diabetes and growth hormone related disorders.

Endocrine Society of Australia **Booth 25**
145 Macquarie Street
Sydney, NSW 2000
Ph: 02 9256 5405, Fax: 02 9251 8174
Contact: Ivone Johnson
Email: esa@racp.edu.au
Web: www.endocrinesociety.org.au
The Endocrine Society of Australia (ESA) is a national non-profit organisation of scientists and clinicians who conduct research and practice in the field of Endocrinology.
The society was founded in 1958 and incorporated in 1986 in the State of Victoria. The Society is governed by the eight members of its Council who are elected every two years by a ballot of the membership in accordance with the Constitution.

Genzyme Australasia **Booth 36**
PO Box 282
North Ryde B/C NSW 1670
Ph: 02 9978 3934, Fax: 02 9889 3900
Contact: Liz Chaffey
Email: elizabeth.chaffey@genzyme.com
Web: www.genzyme.com.au
One of the world's leading biotechnology companies, Genzyme has grown from a small start-up to a diversified enterprise serving patients in 80 countries throughout the world. Genzyme has businesses focused on the needs of patients seen by both private practice and hospital based specialists, including Renal and Transplant Medicine, Enzyme replacement therapies for lysosomal storage disorders, Haematology/Oncology, Endocrinology and Orthopaedics.

IPSEN **Major Sponsor**

Suite 6, 40 Montclair Avenue
Glen Waverley VIC 3150
Ph: 03 8544 8100, Fax: 03 9562 5152
Contact: Briana Romano
Email: info@ipsen.com.au
Web: www.ipsen.com.au

Ipsen Pty Ltd is the Australian affiliate of a global biotechnology specialty care group with a total worldwide staff of more than 4,400. Its strategy is based on fast growing speciality care drugs in oncology, endocrinology, neurology and haematology. Ipsen's Research and Development centres are in Paris, Boston, Barcelona and London.

Since establishing in Australia in 2001, Ipsen has successfully launched Dysport® (botulinum toxin type A) for the treatment of various neuromuscular disorders, Somatuline® Autogel® (lanreotide) for the treatment of acromegaly and the symptoms of carcinoid syndrome and NutropinAq® (recombinant human growth hormone), for growth hormone deficiency in adults and children. In 2009, Ipsen launched Diphereline™ (tripterolin embonate), for the treatment of advanced or metastatic prostate cancer, and began promoting Enablex®* (darifenacin) for the treatment of overactive bladder.

Ipsen delivers customised solutions to healthcare professionals and their patients according to their needs.

* Enablex® is a registered trademark of Novartis Pharmaceuticals Australia Pty Ltd.

Johnson and Johnson **Principal Sponsor, Booth 21**

1-5 Khartoum Road
North Ryde NSW 1670
Ph: 02 9878 9000, Fax: 02 9805 0335
Contact: Sarah Najdek
Email: lyap1@its.jnj.com
Web: www.onetouch.com.au

Johnson & Johnson Medical Pty Ltd is the leading provider of medical devices and support services to the Australian health care system. The company is part of the Johnson & Johnson Family of Companies, one of the world's most comprehensive health care organisations. With its LifeScan diabetes division, J&J is the SMBG leader throughout the USA, Canada and France.

With its LifeScan diabetes division, J&J is the SMBG leader throughout the USA, Canada and France. ONETOUCH® meters have benefited the diabetes community in those countries since 1986. Through its Australian ONETOUCH® launch, J&J seeks to provide a superior product and support offering to benefit the Australian diabetes community.

Mater Pathology **Booth 8**

Level 6, Mater Adult Hospital, Raymond Terrace
South Brisbane QLD 4101
Ph: 07 3163 8111
Contact: Dean Kelly
Email: dean.kelly@mater.org.au
Web: www.mater.org.au

Mater Pathology is Queensland's leading not-for-profit pathology provider and operates a highly sophisticated, state-of-the-art laboratory featuring specialist medical, scientific and technical staff. Mater Pathology offers a comprehensive range of genetic testing for endocrine disorders and is the only laboratory in Australia to offer genetic screening for Maturity Onset Diabetes of the Young.

Medtronic Australasia **Booth 15**

97 Waterloo Rd
Ph: 029857 9000, Fax: 02 9887 1829
Contact: Elmira Leynes
Email: australia.diabetes@medtronic.com
Web: www.medtronic-diabetes.com.au

Medtronic Diabetes is the world leader in insulin pump therapy and continuous glucose monitoring and is firmly committed to helping people living with Type 1 diabetes live healthier lives by providing superior technology and responsive customer support.

Our products include external insulin pumps, related disposable products and continuous glucose monitoring systems.

With our Clinical/Sales Specialist team, 24 hour pump Helpline, large research and development resources in insulin pump therapy and continuous glucose monitoring, Medtronic Diabetes sets the standard in diabetes care.

Visit the Medtronic Diabetes Stand (no. 15) to learn about the latest technologies in Diabetes Management.

Merck Serono **Booth 11**
Unit 3-4, 25 Frenchs Forest Road East
Frenchs Forest VIC PC
Ph: 02 8977 4100, Fax: 02 9975 1516
Contact: Tracey McDonnell
Email: tracey.mcdonnell@merckserono.net
Web: www.merckserono.net
Merck Serono is a division for innovative prescription pharmaceuticals of Merck, a global pharmaceutical and chemical group. Headquartered in Geneva, Switzerland, Merck Serono discovers, develops, manufactures and markets innovative small molecules and biopharmaceuticals to help patients with unmet medical needs.
With annual R&D investment of around € 1billion Merck Serono is committed to growing its business in specialist – focused therapeutic areas including neurodegenerative diseases, oncology, fertility and endocrinology, as well as new areas potentially arising out of research and development in autoimmune and inflammatory diseases.

MSD **Principal Sponsor, Booths27 and 41**
54 - 68 Ferndell Street
South Granville NSW 2142
Ph: 02 9795 9416, Fax: 02 8988 8397
Contact: Michael Bramble
Email: michael_bramble@merck.com
Web: www.msd-australia.com.au
Today's MSD is a global healthcare leader working to help the world be well. MSD is a tradename of Merck & Co., Inc., with headquarters in Whitehouse Station, N.J., U.S.A. Through our prescription medicines, vaccines, biologic therapies, and consumer care and animal health products, we work with customers and operate in more than 140 countries to deliver innovative health solutions. We also demonstrate our commitment to increasing access to healthcare through far-reaching policies, programs and partnerships. MSD. Be well. For more information, visit www.msd-australia.com.au.

Novartis Oncology **Major Sponsor, Booth 26**
54 Waterloo Rd
North Ryde NSW 2113
Ph: 02 9805 3765, Fax: 02 9888 3408
Contact: Nicki Sidwell
Email: nicki.sidwell@novartis.com
Web: www.novartisoncology.com
Novartis Oncology provides a range of innovative therapies and practical solutions that aim to improve and extend the lives of cancer patients. We aspire to develop new medicines that will transform the way cancer is treated, and are therefore committed to ongoing research and development in Australia and New Zealand.

Novo Nordisk **Major Sponsor, Booth 32**
Level 3, 21 Solent Circuit
Baulkham Hills NSW 2153
Ph: 02 8858 3757, Fax: 02 8858 3799
Contact: Kate Alger
Email: kalg@novonordisk.com
Web: www.novonordisk.com
Novo Nordisk is leading the fight against diabetes. Defeating diabetes is our passion and our business.
One of the first companies to introduce insulin, Novo Nordisk is now the world's largest insulin manufacturer and the leading supplier of insulin in Australasia. Our strong commitment to changing diabetes is reflected in our focus on research and development, our partnerships with professional and consumer organisations and our commitment to communities in the developing world through the World Diabetes Foundation.
A world leader in diabetes care, Novo Nordisk is committed to fighting this growing epidemic with the ultimate aim of finding a cure.

Roche Diagnostics **Booth 28**
31 Victoria Avenue
Castle Hill NSW 2154
Ph: 02 8665 9857, Fax: 02 8665 9888
Contact: Sophia Aquino
Email: sophia.aquino@roche.com
Web: www.rochediagnostics.com.au
Roche is a world leader and innovator in medical diagnostics. The Accu-Chek® brand is recognized for over 30 years of quality and innovation in diabetes care worldwide.

Accu-Chek® offers comprehensive diabetes management solutions to healthcare professionals and patients with diabetes. With a top of the range portfolio,
Accu-Chek® offers blood glucose monitors (including the new strip-free system – Accu-Chek® Mobile), insulin delivery systems (including the new Accu-Chek® Combo), lancing devices, diabetes management support systems and professional programmes.
Visit the Accu-Chek® stand to see the exciting new Accu-Chek® products that can help you and your patients better manage diabetes.
Accu-Chek®. Experience what's possible.

Sanofi Aventis **Principal Sponsor, Booth 18**
12-24 Talavera Rd
Macquarie Park NSW 2113
Ph: 02 8666 2000, Fax: 02 8666 3345
Contact: Christine Bell
Email: Christine.Bell@sanofi-aventis.com
Web: www.sanofi-aventis.com
Sanofi-aventis has an 85-year track record of developing effective solutions for diabetes patients, and is a leader in the fight against diabetes.
Our portfolio includes a broad spectrum of therapeutic solutions, with key drugs including insulins and oral hypoglycaemic agents, an exciting pipeline with entirely new classes of pharmaceuticals for use across the diabetes spectrum, and global development partnerships with leading diabetes care organisations. With SoloSTAR® and ClikSTAR® we offer a full range of devices to simplify usage of insulin for patients, by better addressing their different needs and lifestyles.
Sanofi-aventis is making major investments in diabetes treatments, devices and services. We're delighted to be able to support this conference.

Sapphire Bioscience Pty Ltd **Booth 35**
126 Cope Street
Waterloo NSW 2017
Ph: 02 9698 2022, Fax: 02 9698 1022
Contact: Katie Chan
Email: sales@sapphirebioscience.com
Web: www.sapphirebioscience.com
Sapphire Bioscience offers an extensive collection of assay kits, antibodies, inhibitors and receptor agonists/antagonists for assessing endocrine function. Assay kits for adipokines, steroids, neuropeptides, gut peptides, free radical biomarkers, lipid mediators, and environmental stress indicators are available. Other products include transcription factor assays for metabolic and nuclear receptor research.

SciGen (Australia) Pty Ltd **Booth 9**
Suite 1/13B Narabang Way
Belrose NSW 2085
Ph: 02 9485 1800, Fax: 02 9485 1888
Contact: Jean-Claude Balducci
Email: administration@scigen.com.au
Web: www.scigenltd.com
SciGen is a high growth biopharmaceutical company that develops, manufactures and markets biotechnology derived products. SciGen's focus is in the areas of endocrinology, oncology and immunology. Its product portfolio includes vaccines and therapeutics.
The company's portfolio consists of biosimilar products such as human growth hormone, recombinant human insulin, GCSF, EPO, interferon Alfa, and a third generation hepatitis B vaccine. All of SciGen's products have undergone substantial clinical development and trials, and are in the process of securing health registration.

Servier Laboratories **Booth 31**
8 Cato Street
Hawthorn VIC 3122
Ph: 03 8823 7333, Fax: 02 9822 8688
Contact: Deborah Shaw
Email: deborah.shaw@au.netgrs.com
Web: www.servier.com
Servier is a privately owned pharmaceutical company with a long-standing commitment to research and development. In 2005, company founder Dr Jacques Servier revealed that all profit from Servier worldwide operations is now channelled into research and development projects through the recently formed Servier Foundation.

Servier Australia's commercial interests are presently in diabetes (Diamicron 60 MR - gliclazide), disseminated malignant melanoma (Muphoran - fotemustine), postmenopausal osteoporosis (Protos - strontium ranelate) and Cardiovascular disease (Coralan - ivabradine, Coversyl - perindopril arginine, Coversyl Plus - perindopril arginine/indapamide) and most recently Coveram - the combination of perindopril and amlodipine**.**

Society of Reproductive Biology **Booth 22**
Contact: Christopher Grupen
Email: christopher.grupen@sydney.edu.au
Web: www.srb.org.au
The core objective of the Society for Reproductive Biology is to promote the advancement and dissemination of all aspects of basic and strategic science in reproduction, fertility and development, underpinning biomedicine and health, animal production, environment and conservation. It is the premier society of basic and applied reproductive biologists in Australasia, and currently has over 250 members.

Software 4 Specialists Pty Ltd **Booth 40**
PO Box 810
Fairfield NSW 1860
Ph: 1300 133 308
Contact: Dana Segelov
Email: sales@s4s.com.au
Web: www.s4s.com.au
Software 4 Specialists, an Australian company, have designed and developed an innovative clinical software program called Audit4, for Endocrinology/Diabetes. Audit4 is both a complete paperless solution and an audit tool enabling a powerful and instant audit of all aspects of endocrine/diabetes practice. An invaluable tool for MOPS. Clinical practice efficiency is enhanced through electronic tools including automatic express letter to the GP, electronic scripts including streamline authority, investigation requests and downloading results, media manager for organising scanned images/photos and imported documents, instant graphing of pathology results with ability to mark interventions. Links to the front-desk windows-based billing system for patient demographics.

Stratech Scientific APAC **Booth 37**
PO BOX 330
Avalon NSW 2107
Ph: 02 9973 2421, Fax: 02 9012 0019
Contact; Mark Longster
Email: info@stratechscientific.com.au
Web: www.stratechscientific.com.au
Stratech Scientific provides innovative, specialist tools for the Scientific community who need consistent, reproducible results. We cover Salimetrics Salivary Hormone Biomarkers, Poly/Monoclonal Antibodies, Recombinant Proteins, Gold solutions, Genotyping, plus products for working with Proteins. We have built an excellent reputation over 27 years for supplying high quality, competitively priced products. Please visit us on Stand 37.

UpToDate **Booth 6**
PO Box 444
Merrylands NSW 2160
Ph: 02 9689 3888, Fax: 02 96893788
Contact: Maria Raffoul
Email: mraffoul@UpToDate.com
Web: www.uptodate.com
UpToDate is an evidenced based, peer reviewed information resource.
UpToDate answers questions quickly, increases clinical knowledge and improves patient care. Independent studies confirm these benefits. The UpToDate faculty of over 4400 leading physicians writes reviews that include a synthesis of the literature, latest evidence and specific recommendations for patient care.

AUTHOR INDEX

INTRODUCING A NEW WAY FOR PATIENTS TO MEASURE THEIR BLOOD GLUCOSE.

MORE ACCURATELY.

- No blood glucose monitoring system is more accurate than ONETOUCH VERIO®*
- The ONETOUCH VERIO® system is very specific to glucose and has no reaction to maltose and galactose
- In-built no coding means one less step and more convenience
- Easy-to-learn and easy-to-use; an all-in-one system

For further information, please visit us at exhibition stand 21 or at www.onetouch.com.au

ONETOUCH VERIO®

TRI-SURE / STRIP / TECHNOLOGY

*Data on file. When compared against ACCU-CHEK PERFORMER, OPTIUM XCEED, FREESTYLE LITE, ACCU-CHEK ACTIVE, ACCU-CHEK MOBILE. The ONETOUCH VERIO® Blood Glucose Monitoring System is intended to be used for the quantitative measurement of glucose in fresh capillary whole blood. Use only as directed. YOUR HEALTHCARE PROFESSIONAL WILL ADVISE YOU WHETHER THIS PRODUCT IS SUITABLE FOR YOU/YOUR CONDITION. ALWAYS READ THE LABEL. ONETOUCH VERIO® is a registered trademark of Johnson & Johnson. Distributed by: Johnson & Johnson Medical Pty. Ltd. ABN: 85 000 160 403. ACCU-CHEK PERFORMA, ACCU-CHEK and ACCU-CHEK MOBILE are registered trademarks of Roche Diagnostics GMBH. OPTIUM XCEED is a registered trademark of Abbott Laboratories. FREESTYLE is a registered trademark of Abbott Diabetes Care Inc. URSA3177

INVITED ORALS

001

ORIGINS OF DNA DAMAGE IN SPERMATOZOA

R. J. Aitken

Biological Sciences, University of Newcastle, Callaghan, NSW, Australia

DNA damage is frequently encountered in the spermatozoa of sub-fertile male mammals and is correlated with a range of adverse clinical outcomes including impaired fertilization, disrupted embryonic development, increased rates of miscarriage and an enhanced risk of disease in the progeny. The etiology of DNA fragmentation in human spermatozoa is closely correlated with the appearance of oxidative base adducts and evidence of impaired chromatin remodelling during spermiogenesis. In light of these associations we propose a two step hypothesis for the origins of DNA damage in spermatozoa. In Step 1, a variety of intrinsic (diabetes, varicocele, testicular torsion, obesity) and extrinsic (radiofrequency electromagnetic radiation, heat, cigarette smoke, diet, environmental toxicants) factors collude to generate a state of oxidative stress in the testes. This stress impedes spermiogenesis resulting in the generation of spermatozoa with poorly remodelled chromatin. These defective cells readily default to an apoptotic pathway comprising motility loss, caspase activation, phosphatidylserine exteriorization and the production of reactive oxygen species (ROS) by the mitochondria. In Step 2, these mitochondrial ROS attack the spermatozoa inducing lipid peroxidation and oxidative DNA damage, which then leads to DNA strand breakage and cell death. Nucleases activated and released during the apoptotic process are denied access to the sperm nucleus because the unique physical architecture of this cell prevents it. For this reason, a majority of the DNA damage encountered in human spermatozoa is oxidative. Given the importance of oxidative stress in the etiology of DNA damage, there should be a significant therapeutic role for antioxidants in the treatment of this condition. Furthermore, if oxidative DNA damage in spermatozoa is providing a sensitive readout of systemic oxidative stress, the implications of these findings could stretch beyond our immediate goal of trying to minimize DNA damage in spermatozoa as a prelude to assisted conception therapy.

002

PIG SPERM EGG INTERACTION AND FORMATION OF A ZONA PELLUCIDA BINDING COMPLEX

B. M. Gadella

Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Netherlands

In order to achieve fertilization sperm cells first need to successfully interact with the zona pellucida. Before reaching the zona pellucida the sperm cell undergoes extensive remodeling both in the male and female genital tract. These changes serve to mediate optimal recognition of the zona pellucida in the oviduct (primary zona pellucida binding). Optimal sperm-zona interactions are crucial for porcine oocyte fertilization: The zona pellucida- attached sperm cell is triggered to undergo the acrosome reaction and will also become hypermotile. Together these two responses allow the sperm cell to drill through the zona pellucida (secondary zona pellucida binding) and this coincides with local sperm zona drilling so that a few sperm can reach the perivitellin space. This delaying strategy allows only one sperm cell in a given time-point to bind and fuse with the oocyte (fertilization) and thus minimizes the risk of polyspermy. The polyspermy block is essentially executed by the fertilized oocyte that immediately secretes its cortical granule content into the perivitellin space. This content blocks sperm-oocyte fusion either by sticking to the oolemma or by the induction of a biochemical reaction of the zona pellucida (zona pellucida hardening). The cortical reaction thus blocks sperm-zona pellucida binding and/or sperm-zona pellucida drilling. Note that zona pellcuida interactions under pig IVF conditions relatively frequently result in polyspermy. It is not clear whether this is also the case in vivo after natural mating. More importantly we do not know how other artificial reproductive technologies affect polyspermy rates. This is especially relevant for new sperm treatments and insemination technologies in which sperm are activated by capacitation media essentially mimicking the IVF media. Therefore, better understanding of sperm activation and of the arrangement of proteins involved in zona pellucida interactions are relevant for designing strategies to further improve pig reproduction

003

OXIDATIVE STRESS, OSMOTIC STRESS AND APOPTOTIC CHANGES: EFFECTS ON EQUINE SPERMATOZOA

B. A. Ball, S. Meyers

School of Veterinary Medicine, University of California Davis, Davis, CA, United States

Although considerable progress has been made over the past few years in liquid or frozen storage of equine spermatozoa, there remains a large inter-individual difference in the success of semen preservation for the stallion. Equine spermatozoa undergoing low-temperature storage undergo oxidative damage to membrane phospholipids, proteins and chromatin. Osmotic stress also leads to damage to the plasma membrane and alteration in sperm metabolism. Furthermore, evidence from a number of species suggests that ejaculated spermatozoa undergo apoptotic-like changes as a consequence of cryopreservation. It appears likely that these three processes are interlinked and may impact various compartments in the sperm cell via similar pathways. Therefore, an understanding of these processes and their common metabolic pathways may be important in attempts to obviate adverse affects on equine spermatozoa during storage. Further research should

evaluate the molecular pathways which may represent convergence of these stresses on the sperm cell with an aim to reducing their net detrimental effect on sperm during preservation. Supported by the John P. Hughes Endowment, the UC Davis Center for Equine Health, and the National Center for Research Resources, National Institutes of Health.

004

A NEW ERA IN CONTRACEPTIVE DEVELOPMENT: NON-HORMONAL OPTIONS THAT ALSO TARGET SEXUALLY TRANSMITTED INFECTIONS

E. Dimitriadis

Prince Henry's Institute of Medical Research, Melbourne, VIC, Australia

Despite huge increases in access to contraceptives globally more than 700,000 maternal deaths related to unintended pregnancies occurred between 1995 and 2000 mostly in developing countries. Over 80 million women have unintended or unwanted pregnancies annually. Remarkably, there have been no new methods of contraceptives developed in the last 50 years. The extremely high incidence of sexually transmitted infections (STIs) indicates that it is desirable to develop contraceptives that also target STIs. Two interleukin (L) 6-type cytokines, leukemia inhibitory factor (LIF) and IL11, are obligatory for implantation in mice and are dysregulated in endometrium of some women with infertility. Both LIF and IL11 are also thought to have roles in Chlamydia-induced inflammation which can lead to a multitude of pathologies. LIF and IL11 antagonists were produced and their contraceptive efficacy tested in mice. Polyethylene glycol (PEG) was conjugated to LIF antagonist (LA) or IL11 antagonist (IL11A) to increase their serum half-life. PEGLA injected during the peri-implantation period blocked LIF action in the endometrium and totally prevented embryo implantation while having no embryo toxic effects[1]. Similarly, injection of PEGIL11A blocked decidua formation resulting in pregnancy failure[2] . In women, vaginally administered drugs preferentially localise to the uterus suggesting that vaginal administration of PEGLA is an appropriate delivery method for contraceptive purposes. Further, vaginally administered PEGLA may be useful as a 'dual-role' contraceptive to also block STIs. PEGLA administered via vaginal gel was shown to prevent implantation having minimal effects on non-uterine LIF targets. This is the first study to show the contraceptive efficacy of a PEGylated compound delivered vaginally. It further indicates that PEGLA may be useful as a dual-role contraceptive. Contraceptive trials in non-human primates are currently underway to determine the effect of PEGLA on implantation. If effective, this will offer new opportunities as pharmacological, non-hormonal dual-role contraceptives for women.

(1) White CA, Zhang JG, Salamonsen LA, Baca M, Fairlie WD, Metcalf D, Nicola NA, Robb L, Dimitriadis E (2007) Proc Natl Acad Sci USA.104:19357-62

(2) Menkhorst E, Salamonsen LA, Robb L, Dimitriadis E (2009) Biol of Reprod. 80: 920-927

005

PHEOCHROMOCYTOMA AND PARAGANGLIOMA: NEW DISCOVERIES TO GUIDE CLINICAL PRACTICE AND FUTURE RESEARCH

K. Pacak

Section on Medical Neuroendocrinology, NICHD, Bethesda, MD, United States

Pheochromocytomas (PHEO) are catecholamine-producing tumors most commonly located in the adrenal gland and less frequently in extra-adrenal locations (known as paragangliomas; PGL). Several outstanding discoveries have recently been made that have substantially improved our knowledge of the pathogenesis, genetics, diagnosis, localization, and treatment of these tumors. Three new genes (SDHAF2, TMEM127, and SDHA) were discovered to play a role in the pathogenesis of these tumors, though much less frequently than the other well known PHEO susceptibility genes (MEN2, NF1, VHL, SDHB/C/D). Gastrointerstinal stromal tumors (GIST) have been found to be associated with the presence of succinate dehydrogenase subunit B (SDHB) gene mutation and PGLs. The loss of immunohistochemistry for SDHB was found to "mark" the presence of SDHx related PHEO and PGL and can be used to triage proper genetic testing. New genes, SNAIL and carboxipeptidase A, have been implicated in the pathogenesis and prediction of metastatic or recurrent disease. SDHB gene mutations have been implicated as the most common cause of malignant PHEOs and PGLs in both children and adults. Current observations suggest that familial tumors may be twice as common in children than adults. Mediastinal and Zuckerkandl organ PGLs are most commonly related to SDHx gene mutations. It has been proposed that all patients with metastatic PHEOs and PGLs should first undergo SDHB genetic screening while those with multiple PGLs, including head and neck tumors, should undergo SDHD genetic screening as well as SDHB screening. Patients with bilateral adrenal PHEOs should undergo genetic screening for MEN2 or VHL guided by syndromic presentation, family history, or biochemical phenotype (VHL tumors almost always presents with a noradrenergic phenotype). Genetic screening for NF1 is not recommended since syndromic presentation is usually very characteristic, and despite a similar biochemical phenotype to MEN2 (both have adrenergic biochemical phenotype), clinical presentation and family history distinguishes these two types very well. Children carrying the SDHB gene mutation should be screened starting at age five with plasma or urine metanephrines tested. Screening using imaging studies can be implemented later in life or if biochemical results draw suspicion for a tumor. Experts in the field agree on using either plasma or urine metanephrine analysis as the first test in the biochemical diagnosis of these tumors. The marker for dopamine secreting tumors (usually related to SDHB gene mutations and clinically "silent" tumors) is methoxytyramine. In more than 99% of patients, the measurement of plasma metanephrines and methoxytyramine can distinguish MEN2 and NF1 patients from VHL and SDHx patients. The majority of VHL and SDHx patients can be well distinguished based on plasma methoxytyramie measurements. The clonidine test is highly recommended in patients where the diagnosis is equivocal e.g. the elevation of plasma metanephrines is less than about 4 times above the upper reference limit; the glucagon test has been abandoned due to its low sensitivity. Functional imaging using positron emission tomography compounds (PET) should become a gold standard in the localization of these tumors. [^{18} F]-fluorodopa PET is the best imaging modality for head and neck PGLs, [^{18} F]-fluorodopamine PET is the best for

sympathetic (outside head and neck) PHEOs and PGLs, with the exception of SDHB related metastatic tumors where [^{18} F]-fluorodeoxyglucose PET is recommended. [^{123} I]-MIBG is not recommended for the initial localization of these tumors, except adrenal PHEOs and situations when [^{131} I]-MIBG treatment is considered (e.g. metastatic disease). A new highly specific activity compound Ultratrace™ [^{131} I]-Iodobenguane (Azedra) is an approach promising for the treatment of metastatic PHEO and PGL. Adrenoceptor blockade before CT with contrast is no longer recommended. Radiofrequency ablation is a minimally invasive and recommended procedure for the treatment of metastatic organ and bone lesions. Laparoscopy and cortical-sparing surgery are attractive and well-accepted surgical approaches. Current data suggests there is no survival benefit between CVD chemotherapy treated and untreated patients, although CVD chemotherapy can be very beneficial initially in patients with SDHB related metastatic PHEOs and PGLs. Therapeutic options includng stabilization of HIF1alpha and topoisomerase inhibitors are new treatment avenues.

(1) This research was supported by the Intramural Research Program of the NICHD/NIH.

006

HUMAN GERM CELL FORMATION AND DIFFERENTIATION FROM PLURIPOTENT STEM CELLS

R. A. Reijo Pera

Center for Human Embryonic Stem Cell Research and Education, School of Medicine, Stanford University, Stanford, United States

Human embryo development begins with the fusion of egg and sperm, followed by reprogramming of the DNA, a series of cell divisions and activation of the embryo's genome. As development continues, the germ cells (egg and sperm) must be set aside from other cell types. A major cause of infertility in men and women is quantitative and qualitative defects in human germ cell (oocyte and sperm) development. Yet, it has been difficult to study human germ cell development, especially features that are unique relative to model organisms. We have developed a system to differentiate human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) to germ cells and to quantitate and isolate primordial germ cells (PGCs) derived from both XX- and XY-bearing hESCs and iPSCs. This allowed silencing and overexpression of genes that encode germ cell-specific cytoplasmic RNA-binding proteins (not transcription factors) and resulted in the modulation of human male and female germ cell formation and developmental progression. We observed that human *DAZL (Deleted in AZoospermia-Like)* functions in female and male PGC formation and maintenance, whereas closely-related family members, *BOULE* and *DAZ*, promote entry into meiosis and development of haploid gametes with sperm-specific methylation patterns at imprinted loci in the male. We also conducted critical proof-of-concept studies in mice that showed that phenotypes observed in germ cell development *in vitro* from wildtype, heterozygous, and *Dazl-/-* mutation-carrying mouse ESCs (mESCs) mirrored the phenotypes that were observed *in vivo*. Furthermore, transplantation of XX mESC-derived oocytes resulted in recruitment of somatic cells to form follicles. These studies comprised the first direct experimental analysis of the genetics of human germ cell development and set the stage for extensive exploration of complex genetic variants linked to infertility. Results are significant to the generation of gametes for developmental genetic studies and potential clinical applications.

007

RESEARCH LEADERSHIP

J. Shine

Garvan Institute, Darlinghurst, NSW, Australia

Research leadership - a discussion on some of the ingredients that are involved in managing research teams and achieving successful research outcomes in a highly competitive environment. The rationale behind career choices, researcher motivation and selection of research areas will be explored.

008

N-3 LONG CHAIN POLYUNSATURATED FATTY ACIDS: THE GOOD OILS FOR PREGNANCY OUTCOME?

M. Makrides[1,2]

[1]Child Nutrition Research Centre, Women's and Children's Health Research Institute, Adelaide, SA, Australia
[2]Paediatric and Reproductive Health, University of Adelaide, Adelaide, SA, Australia

The metabolic demand for docosahexaenoic acid (22:6 n-3, DHA) is increased during pregnancy because of the extra needs of the fetus, expanded maternal cell mass and placenta. However, in Western countries maternal dietary DHA intake in pregnancy is low and it is not clear whether adaptive metabolic mechanisms, such as increased DHA synthesis from precursor fatty acids, are capable of meeting the increased DHA need in pregnancy. Consequently randomized controlled trials are important to determine whether additional dietary DHA in pregnancy modifies maternal or infant health outcomes. The available randomized comparisons of DHA supplements, largely as fish oil, vs placebo have demonstrated a modest overall increase in the duration of gestation. Although this increase in gestation does not always translate to a reduction in the risk of preterm birth, a recent meta-analysis suggests that fish oil supplementation reduces the risk of early preterm birth (<34 weeks gestation). Other, more contemporary, trials have assessed outcomes as diverse as maternal depression, infant

visual acuity and development, and infant growth and allergy. The outcomes of these newer trials have not been conclusive because they have often been limited by small sample size. However, large-scale trials with longer term maternal and childhood outcomes are in progress in Australia, Europe and USA.

009

MATERNAL ETHANOL CONSUMPTION ALTERS THE EPIGENOTYPE AND PHENOTYPE OF OFFSPRING IN A MOUSE MODEL

N. Kaminen-Ahola[1], A. Ahola[1,2], M. Maga[3], T. Cox[3], E. Whitelaw[1], S. Chong[1]

[1]Epigenetics Laboratory, Queensland Institute of Medical Research, Herston, QLD, Australia
[2]Department of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland
[3]Division of Craniofacial Medicine, University of Washington, Seattle, United States

Exposure to environmental triggers such as poor nutrition or high levels of alcohol *in utero* can lead to compromised foetal development and adult disease in humans. The underlying mechanisms remain unknown. We have developed a model of moderate ethanol exposure in the mouse based on maternal ingestion of 10% ethanol from fertilisation to mid-gestation. This strategy produces offspring with craniofacial and growth restriction phenotypes that are reminiscent of foetal alcohol syndrome in humans. We also observe increased DNA methylation and transcriptional silencing at an epigenetically sensitive allele, *Agouti viable yellow*, which is linked to changes in mouse coat colour. Our results show that ethanol can affect adult phenotype by altering the epigenotype of the early embryo. Interestingly, maternal ethanol consumption prior to conception had a similar effect on *Agouti viable yellow*, suggesting that ethanol-induced epigenetic changes can also occur in maturing oocytes. Future work is directed towards genome-wide DNA methylation and gene expression analyses as well as further characterisation of foetal alcohol syndrome-like phenotypes in our mouse model.

010

PERICONCEPTIONAL UNDERNUTRITION: LIFE-LONG EFFECTS FOR THE OFFSPRING

F. H. Bloomfield[1,2], M. H. Oliver[1,2], A. L. Jaquiery[1,2], C. Hernandez[1,2], J. R.G. Challis[4], A. White[3], J. E. Harding[1]

[1]Liggins Institute, University of Auckland, Auckland, New Zealand
[2]National Research Centre for Growth and Development, New Zealand
[3]Faculty of Life Sciences, University of Manchester, Manchester, United Kingdom
[4]Physiology, University of Toronto, Toronto, Ontario, Canada

Poor nutrition in women of child-bearing age is common, even in Western countries. It has been estimated that approximately 30% of women of child-bearing age in affluent cities such as Sydney and Southampton are either actively dieting or have a nutritional intake that does not meet daily recommended requirements for all nutrients. We have investigated the effect of reduced maternal nutrition before and around the time of conception on fetal growth and development, and have followed offspring through to adulthood. In this paradigm, ewes were fed to lose 10-15% of their body weight and then to gain weight according to conceptus mass. Control ewes were well fed throughout. Different timing and duration of undernutrition in the periconceptional period were utilised to investigate the most critical window for fetal development. Periconceptional undernutrition resulted in accelerated development of the fetal hypothalamic-pituitary-adrenal (HPA) and glucose-insulin axes in late gestation, and preterm birth. Offspring of periconceptionally undernourished ewes demonstrated altered laterality and an altered response to isolation stress; HPA axis function was also suppressed. As offspring aged, glucose tolerance decreased and became significantly impaired by young adulthood compared with control offspring. The effects of maternal undernutrition on offspring were modified by offspring sex and also by being one of a twin pair. Interestingly, our data also demonstrate that conception as a twin, regardless of maternal nutritional status, also affects all these outcomes but in a different way to maternal undernutrition. Preliminary data suggest that epigenetic changes in feeding centres of the hypothalamus may play a role in the mechanism behind some of these effects. These studies suggest that even moderate maternal undernutrition in very early pregnancy has life-long effects. Should this also be true in humans, then health care messages for women may need to be targeted prior to pregnancy.

011

GESTATIONAL DIABETES – COMPLICATIONS, MANAGEMENT, OUTCOMES

J. A. Rowan

National Women's Health, Auckland City Hospital, Grafton, New Zealand

Gestational diabetes (GDM) is associated with increased maternal risks of hypertensive complications, caesarean section and later diabetes. The fetus is exposed to an excess nutrient load and risks of macrosomia, trauma at delivery, neonatal complications and later obesity and associated metabolic consequences. Perinatal outcomes are improved by treating GDM, but the effect on longer term outcomes is not clear. Can we improve pregnancy and later outcomes further by considering choice of medication and treatment targets?

The metformin in gestational diabetes (MiG) trial demonstrated that pregnancy outcomes were not different between women randomized to metformin compared to insulin. A composite of neonatal complications was seen in 32.0% and 32.2% respectively, RR 0.99 (95% CI 0.80-1.23). Examining glucose control during treatment in tertiles, women who achieved a mean fasting capillary glucose level <4.9 mmol/l had the lowest risk of neonatal complications. Those achieving a postprandial capillary glucose mean <6.5mmol/l had lower rates of preeclampsia and birth weight >90th centile. Obesity was not a significant factor predicting outcomes (unlike diet-alone treated women).

Preliminary analyses from the follow up of two year old children from the MiG trial, the offspring follow up (TOFU) show body composition, diet and activity assessments are similar, with small differences between the metformin and insulin groups, respectively in biceps (6.1 vs 5.6 mm p = 0.04) and subscapular skin folds (6.38 vs 6.10 mm p=0.03) and upper arm circumference (17.3 vs 16.7 cm p=0.003). Ratios of central to peripheral fat as measured by waist: hip circumference, suscapular:triceps skin folds and abdominal:thigh fat by DEXA were no different. Further analyses will be performed when the final data entries are completed and details of these will be presented.

012

DOES THE PATTERN OF GLUCOCORTICOID SECRETION MATTER?

S. Lightman

Laboratories for Integrative Neuroscience and Endocrinology (LINE), University of Bristol, Germany

If glucocorticoids are released in pulses, how then have peripheral tissues evolved to read these digital signals? At the single cell level, we see very rapid and transient binding of GR to GREs. This rapidity of exchange on and off the DNA is only seen for the native ligands cortisol and corticosterone, but not any of the synthetic glucocorticoids. In the liver and in the hippocampus, each pulse of DNA binding is associated with a pulse of gene transcription and we find differential effects on different glucocorticoid responsive genes. Disease is associated with altered patterns of presentation of ligand and I shall discuss the effect of inflammatory arthritis in the rodent and obstructive sleep apnoea in man. Furthermore, changes in body temperature alter the affinity of CBG, resulting in marked changes in the dynamics of pulses of free hormone.

013

MICROCIRCULATION AND ENDOCRINE SYSTEMS: IMAGING PITUITARY FUNCTION AT CELLULAR RESOLUTION *IN VIVO*

P. Le Tissier

Division of Molecular Neuroendocrinology National Institute for Medical Research Mill Hill London, United Kingdom

The proper functioning of the pituitary gland is vital for the regulation of many important body functions. The secretion of hormones is associated with a high metabolic cost; not only do the energy demands of thousands of highly active endocrine cells have to be met, but the temporally precise entry of hormone into the bloodstream also requires large-scale changes in vascular dynamics. In order to achieve this fine balance between energy supply and hormone uptake, the pituitary gland is highly vascularized by fenestrated capillaries which deliver nutrients, oxygen and stimuli, absorb secreted cell products, and deliver hormone to peripheral target tissues such as the liver or gonads. Since the generation of an appropriate physiological response by peripheral tissue relies on the message encoded by pulsatile secretion of hormone, organisms have evolved mechanisms to tightly regulate blood perfusion and oxygenation. This allows pituitary endocrine cell activity and output to be temporally correlated with changes in blood flow, resulting in the replenishment of cell energy stores, the coordinated passage of hormone into the vasculature and the efficient generation of hormone pulses. Furthermore, since the pituitary gland must display marked plasticity in response to acute and chronic physiological demand, these mechanisms must rapidly and reversibly adapt to the prevailing conditions. The aim of the lecture is to describe the mechanisms which exist in endocrine systems to facilitate coordinated cell activity and output, and the role that these mechanisms play during periods of plasticity in pituitary hormone release. To do so, new *in vivo* approaches (cellular in vivo imaging) were developed to measure local blood flow, oxygen partial pressure and cell activity at single-cell resolution in mouse pituitary glands *in situ* (1). Placing emphasis on the pituitary gland which, via its hormonal output, dictates important homeostatic responses ranging from growth and metabolism to other basic body functions, we will discuss the importance of the interplay between blood flow regulation and oxygen tensions as well as the role of the perivascular space in pituitary gland function under both normal and pathological conditions.

(1) 1- Lafont C, Desarménien MG, Cassou M, Molino F, Lecoq J, Hodson D, Lacampagne A, Mennessier G, El Yandouzi T, Carmignac D, Fontanaud P, Christian H, Coutry N, Fernandez-Fuente M, Charpak S, Le Tissie

014

PULSATILE GNRH SECRETION

I. Clarke

Department of Physiology, Monash University, Clayton, VIC, Australia

Gonadotropin releasing hormone (GnRH) is the primary driver of reproduction. This brain neuropeptide controls the synthesis and secretion of gonadotropins from the pituitary gland through its cognate GPCR. Modulation of the secretion and action of GnRH is by various other brain systems as well as feedback action of sex steroids from the gonads. How various factors such as stress, nutrition, season and emotive states regulate/modulate GnRH function has been the subject of intense study, but the key elements that convey information to GnRH cells have only recently been identified. Amongst these regulators, kisspeptin plays a major role, transmitting the feedback effects of gonadal steroids. This paper will discuss the possible origins of pulsatile GnRH secretion and whether this is a property innate to the GnRH neurons themselves. Examples of modulation of GnRH and luteinising hormone (LH) pulse frequency and amplitude will be presented.

015

THE SMELL OF SEX – PHEROMONES AND GNRH SECRETION

P. Hawken, G. Martin

School of Animal Biology, University of Western Australia, Nedlands, WA, Australia

Chanel No.5, Emporio Armani, Old Spice… as humans we spend millions of dollars each year trying to find the perfect scent to attract the opposite sex, but perhaps we should take a lesson from the animal kingdom and let our natural scent do the talking. Male pheromones have an almost instantaneous effect on the hypothalamic–pituitary axis and elicit ovulation in seasonally and pre-pubertally anovulatory animals. Much of the work in this field has used large animal models, with pulsatile LH secretion as a bioassay to profile the effects of male pheromones on GnRH output (Review: Delgadillo et al., 2009). The validity of this approach is supported by work in goats showing that the LH response of females to male pheromones is at least partly due to stimulation of the GnRH pulse generator (Hamada et al., 1996). Furthermore, the GnRH neurons predominantly responsible for mediating the dynamic increase in pulsatile LH secretion are located in the pre-optic area of female sheep (Gelez and Fabre-Nys, 2006). The male pheromone that drives this phenomenon appears to encode specific information about male identity with only unfamiliar or 'novel' males capable of increasing GnRH output. Reception of the pheromone is thus linked memory centres in the hippocampus where, within a couple of hours, it evokes cell division, probably neurogenesis that is linked to learning and memory (Hawken et al., 2009).

Female 'pheromones' have been studied much less but, in male sheep, they activate GnRH neurons in the medio-basal hypothalamus and increase LH secretion (Review: Delgadillo et al., 2009). Intriguingly, female pheromones are also thought to be responsible for synchronising menstruation in women, but the role of GnRH secretion in this phenomenon is not fully understood. In conclusion, pheromones clearly have a profound affect on reproduction in many species and perhaps we have underestimated their role in human attraction.

(1) Delgadillo JA et al. (2009) Behav Brain Res 200:304-14.
(2) Gelez H, Fabre-Nys C (2006) Neuroscience 140:791-800.
(3) Hamada T et al. (1996) Neuroendocrinology 64:313–9.
(4) Hawken PAR et al. (2009) Biol Reprod 80:1146-51.

016

MANAGEMENT AND LONG TERM CONSEQUENCES OF SUBCLINICAL THYROID DISEASE

J. Franklyn

School of Clinical and Experimental Medicine College of Medical and Dental Scien, University of Birmingham, Edgbaston, BM, United Kingdom

Subclinical hyperthyroidism is characterised by low serum TSH with normal free T4/T3. It may reflect early Graves' disease or toxic nodular hyperthyroidism, and is found in 5% of the over 60's. About 20% of those taking T4 have low TSH, indicating mild over-treatment. In those not prescribed T4 it is important to exclude causes of low TSH such as non-thyroidal illness and drugs. The potential risks of subclinical hyperthyroidism are cardiovascular and osteoporosis. Effects on cardiac function are well documented, as are increased risk of AF and vascular mortality. Bone mineral density may be reduced, especially in post menopausal women, perhaps with increased fracture risk. There are no treatment studies with clinical endpoints, although some evidence of improvement in BMD. Guidelines favour treatment in the elderly with proven early hyperthyroidism, especially with AF or vascular risk. *Subclinical hypothyroidism* is high TSH with normal free T4. If a standard cut off for TSH of 4.5mU/L is adopted, the prevalence is 10% in the over 60's and rises with age. It is also found in 25% of those taking T4. Possible associations include hyperlipidaemia, vascular risk, impaired cognitive function, and impaired well being. Epidemiological studies provide conflicting evidence regarding vascular risk but there may be an association with ischaemic heart disease and heart failure in those with TSH >10mU/L. There is a lack of association with changes in cognitive function, neuropsychiatric symptoms or well being. Most stu dies of T4 treatment show minor effects on lipids and minimal/no effect on cognitive function/symptoms. The main indication for treatment is therefore risk of progression to overt hypothyroidism. Guidelines suggest T4 treatment in those with TSH >10mU/L. An exception to this is pregnancy in which even mild subclinical hypothyroidism (TSH 5-10mU/L) should be treated because of possible association with effects on neurodevelopment in offspring.

017

WHAT IS NORMAL THYROID FUNCTION, ANYWAY?

J. Walsh

Department of Endocrinology & Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia

In order to diagnose thyroid dysfunction, clinicians need to know what normal thyroid function is, but defining this turns out to be rather difficult. In general, serum TSH is a more sensitive marker of thyroid dysfunction than is free T4, but the reference range for TSH remains highly contentious, despite a wealth of normative data. Normal thyroid function can also be defined by health outcomes (such as symptoms of ill health or cardiovascular outcomes) which are associated with different degrees of thyroid dysfunction), and evidence for this will be reviewed. The key concept of the existence of individual setpoints for pituitary-thyroid axis function will be emphasised, and novel data regarding the genetic basis of this will be presented.

018

IODINE DEFICIENCY IN AUSTRALIA AND OVERSEAS

C. J. Eastman

AM MD, NSW, Australia

Adequate maternal iodine intake is essential during pregnancy to ensure normal brain development in the offspring. At a global level, iodine deficiency is the leading cause of preventable mental handicap in our world. At an individual level, the developing brain is extremely vulnerable to even minor degrees of maternal hypothyroxinemia secondary to iodine deficiency. Even mild, clinically unrecognisable, hypothyroxinemia can cause foetal wastage from miscarriage or premature labour or if the foetus survives there may be serious irreversible neuromotor deficits rendering a child handicapped for life. Metanalyses of the effects of population iodine deficiency studies show an average loss of 13.5 IQ points in the offspring of moderately to severely iodine deficient mothers. The consequences of mild iodine deficiency are less certain. The invisibility of iodine deficiency, particularly when it is mild to moderate, makes it all the more dangerous. Despite enormous efforts by the ICCIDD and UN Agencies, WHO estimates that almost 2 billion people worldwide, comprising over 300 million children in 54 countries, still have inadequate iodine intake. More than 41 million newborns each year are not protected from iodine deficiency.

National surveys have shown that iodine deficiency has re-emerged as a significant public health problem in Australia. This is the outcome of a drastic decline in iodine content of dairy products and poor household usage of iodised salt. We estimate the average intake of iodine in pregnant Australian women to be (132 ug per day), which is approximately half the WHO/UNICEF/ICCIDD recommended intake of >250 ug per day. Given these findings, there is an urgent need to address this problem to protect the brains of the next generation of Australian children.

019

GENETIC VARIATION AND THYROID HORMONE FUNCTION

V. Panicker[1,2]

[1]*Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia*
[2]*Henry Wellcome Laboratories for Integrative Neurosciences and Endocrinology, University of Bristol, Bristol, United Kingdom*

It has been clearly shown that an individual's thyroid hormone and TSH levels vary little over time, whilst there is greater variation between individuals. Furthermore, twin and sibling studies have confirmed that this individual 'set point' is predominantly genetically determined. In recent years, advances in genetic technology have enabled us to investigate which genes are responsible.

Both candidate gene studies and genome-wide association studies (GWAS) have identified thyroid hormone and TSH associations. Candidate gene studies have revealed the effect of polymorphisms in the iodothyronine deiodinase 1 gene (*DIO1*) on free T4 and free T3 [1] and polymorphisms in the TSH-receptor gene (*TSHR*) on TSH [2]. GWAS have revealed polymorphisms influencing two genes that are associated with TSH concentrations; phosphodiesterase 8B (*PDE8B*) [3] and capping protein muscle z-line beta (*CAPZB*) genes [4]. Individually these are responsible for a very small amount of the variation in thyroid hormone and TSH concentrations (1-2% each); hence these phenotypes are likely to be polygenically derived. Further, larger GWAS are currently being undertaken and there will undoubtedly be further genes to add to this list soon.

Influencing serum thyroid hormone and TSH concentrations is not the only way genetic variation can affect thyroid hormone action. Given the complex path by which thyroid hormones exert their actions within cells, it is reasonable to expect that polymorphisms in the genes involved in this pathway could influence clinical phenotypes without necessarily altering serum levels. This has been shown already with polymorphisms in the iodothyronine deiodinase 2 gene (*DIO2*) associated with such diverse phenotypes as osteoarthritis [5], mental retardation [6] and psychological well-being [7], and polymorphisms in *TSHR* being associated with bone density [8] and insulin resistance [9].

Discovery of these polymorphisms has increased our understanding of thyroid hormone action, 'normal' thyroid hormone levels and in the future may provide targets for new treatments for thyroid and metabolic disease.

(1) Panicker V, Cluett C, Shields B, Murray A, Parnell KS, Perry JR, et al. J Clin Endocrinol Metab 2008;93(8):3075-81
(2) Hansen PS, van der Deure WM, Peeters RP, Iachine I, Fenger M, Sorensen TI, et al. Clin Endocrinol (Oxf) 2007;66(6):827-32
(3) Arnaud-Lopez L, Usala G, Ceresini G, Mitchell BD, Pilia MG, Piras MG, et al. Am J Hum Genet 2008;82(6):1270-80
(4) Panicker V, Wilson SG, Walsh JP, Richards JB, Brown SJ, Beilby JP et al. submitted
(5) Meulenbelt I, Min JL, Bos S, Riyazi N, Houwing-Duistermaat JJ, van der Wijk HJ, et al. Hum Mol Genet 2008;17(12):1867-75
(6) Guo TW, Zhang FC, Yang MS, Gao XC, Bian L, Duan SW, et al. J Med Genet 2004;41(8):585-90

(7) Panicker V, Saravanan P, Vaidya B, Evans J, Hattersley AT, Frayling TM, et al. J Clin Endocrinol Metab 2009;94(5):1623-9
(8) van der Deure WM, Uitterlinden AG, Hofman A, Rivadeneira F, Pols HA, Peeters RP, et al. Clin Endocrinol (Oxf) 2008;68(2):175-81
(9) Peeters RP, van der Deure WM, van den Beld AW, van Toor H, Lamberts SW, Janssen JA, et al. Clin Endocrinol (Oxf) 2007;66(6):808-15

020

PATHOGENESIS OF THYROID DISEASES

J. Franklyn

School of Clinical and Experimental Medicine College of Medical and Dental Scien, University of Birmingham, Edgbaston, BM, United Kingdom

Thyroid disorders, particularly autoimmune hyper- and hypothyroidism, are common and often cluster in families. Recent large scale genetic studies have identified key genes contributing to genetic susceptibility to autoimmune thyroid disease, including HLA, CTLA4 and the TSH receptor. Perturbations of thyroid status, and of thyroid hormone action, in the pregnant woman or in the fetus significantly affect fetal development, including development and function of the placenta and brain. A variety of factors contribute to development of goitre and thyroid cancer. Novel insights into the function within thyroid cells of the human securin PTTG, and its binding partner PBF, together with understanding of their influence on the function of the sodium iodide symporter (responsible for iodide uptake into thyroid cells) provide some new insight into goitre and thyroid cancer pathogenesis and potential impact on radioiodine treatment.

021

HORMONAL REGULATION OF SPERMATOGENESIS

D. Handelsman

ANZAC Research Institute, The University of Sydney, NSW, Australia

Spermatogenesis is a spatially and temporally co-ordinated proliferation of the germinal epithelium in the semeniferous tubules. The germ cells are embedded in a scafolding formed by adjacent Sertoli cells linked tightly by intercellular junctions and with each germ cell enshrouded by elongations of Sertoli cell cytoplasm. Spermatogenesis comprises serial stages from the mitotic replication of the stem and early germ cells, followed by meiosis, the reductive division producing haploid, amorphous gametes which subsequently undergo spermiogenesis, the metamorphosis into terminally differentiated and functional spermatozoa. Although long known that all but the earliest stages are hormonally regulated by pituitary secretion of LH and FSH, it has remained difficult to separate gonadotrophin effects by classical endocrine ablate-replace methods as these two heterodimeric hormones have identical a and homologous β subunits, are secreted from the same pituitary gonadotrophs to target cognate receptors expressed on adjacent testicular cells as equally homologous, heptahelical G-coupled protein receptors. Over two decades our laboratory has developed a variety of complementary genetic and pharmacological approaches to dissect the individual and co-operative effects of LH, its main effector testosterone and FSH on spermatogenesis. Using the gonadotrophin and testosterone deficient *hpg* mouse, double transgenic human FSH secreting mouse and the androgen receptor knockout mouse lines together with steroidal depot homone delivery, we have explored systematically and defined the individual primary actions of FSH and testosterone and their interactions in the regulation of testis growth, spermatogenesis and ultimately male fertility.

022

TARGETING THE CANCER-PERITONEAL INTERACTION IN OVARIAN CANCER

C. Ricciardelli[1], M. P. Ween[1], N. A. Lokman[1], R. J. Rodgers[1], P. Hoffmann[3], M. K. Oehler[1,2]

[1]Robinson Institute and School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, Australia
[2]Gynaecological Oncology, Royal Adelaide Hospital, Adelaide, SA, Australia
[3]Adelaide Proteomics Centre, University of Adelaide, Adelaide, SA, Australia

Ovarian cancer metastasis is characterized by the shedding of malignant cells from the surface of the ovary and their implantation onto the peritoneal surface which lines the abdominal cavity. As the factors promoting this process are poorly understood, we investigated the ovarian cancer–peritoneal interaction by means of *in vitro* co-culture experiments with ovarian cancer (OVCAR-5 and SKOV-3) and peritoneal (LP-9) cells. Two of the proteins differentially expressed in the co-culture secretome identified by mass spectrometry include the extracellular matrix protein transforming growth factor-beta-induced protein (TGFBIp, also known as big-H3) and the calcium-phospholipid binding protein, annexin A2. Our recent studies have demonstrated that TGFBIp is abundantly expressed by peritoneal cells and treatment with recombinant TGFBIp could significantly increase the motility and invasion of ovarian cancer cells (OVCAR-5 and SKOV-3) and their adhesion to peritoneal cells (OVCAR-5, SKOV-3 and OVCAR-3) [1]. Furthermore, we found that TGFBIp undergoes processing in the ovarian cancer–peritoneal cell co-culture via the protease plasmin. We have additionally characterized the expression of annexin A2 protein in human ovarian cancer tissue and cell lines and investigated the role of annexin A2 in ovarian cancer metastasis. We observed higher annexin A2 expression in the highly metastatic OVCAR-5 cells compared to less metastatic OVCAR-3 cells and increased levels of annexin A2 in ovarian cancer cells adjacent to peritoneal cells. In addition, treatment with neutralizing annexin A2 antibodies could inhibit OVCAR-5 cell motility, invasion and adhesion to the peritoneal cells. These findings add to our understanding of the

interaction between ovarian cancer and peritoneal cells and suggest that both TGFBIp and annexin A2 are part of a tumor-host signal pathway between ovarian cancer and peritoneal cells that could be exploited as novel therapeutic targets.

[1] Ween MP, Lokman, NA, Hoffmann P, Rodgers RJ, Ricciardelli C & Oehler MK . Transforming growth factor-beta induced protein (TGFBIp) secreted by peritoneal cells increases the metastatic potential of ovarian cancer cells *Int J Cancer*, 2nd June 2010, epub ahead of print).

023

ENDOCRINE TUMOUR GENETICS - ADVANCES IN DIAGNOSIS AND TREATMENT

B. G. Robinson

Kolling Institute of Medical Research & Department of Endocrinology, Royal North Shore Hospital and University of Sydney, NSW, Australia

The molecular basis of a number of endocrine disorders is now known and translated to clinical management for many inherited endocrine tumour syndromes. Genetic testing for germline mutations in the following genes is now widely available: *RET* proto- oncogene in multiple endocrine neoplasia type 2; *VHL* in von Hippel Lindau disease, *MEN 1* in endocrine neoplasia type 1; *HRPT2* in hyperparathyroidism – jaw tumour syndrome; succinate dehydrogenease subunit B (*SDHB*), subunit C (*SDHC*) and subunit D (*SDHC*) in familial paraganglioma/phaeochromocytoma syndromes; *PTEN* in Cowden's syndrome; and the calcium-sensing receptor gene (*CASR*) in familial hypocalciuric hypercalcaemia. Once a mutation has been identified in the index case leading to a diagnosis, this indicates not only regular screening (biochemical and radiological) for associated tumours but also the offering of genetic testing to all first degree relatives to ascertain the presence or absence of the specific family mutation. New imaging techniques for detection of primary tumours and metastases are continually being developed but may not be widely available yet. While surgical resection remains the primary treatment in many cases, in these endocrine tumour syndromes, therapy for malignant disease is generally ineffective. Based upon advances in the identification of pathways such as tyrosine kinase cell signalling, and involvement in tumour formation, specific drug therapies can now be used.

024

TOWARDS RNAI THERAPY FOR CANCER – SOLVING THE CRITICAL ISSUES

N. A.L. McMillan

UQ Diamantina Institute and School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, QLD, Australia

RNA Interference holds great promise as a therapy for viral diseases via its ability to directly kill infected cells via down-regulation of critical target genes. However, to be effective it must overcome the issues of delivery and efficacy that currently limit its clinical adoption. We have been addressing these two issues over the last few years. *In vivo* delivery is clearly holding up adoption with large, positively-charged and unstable siRNA molecules difficult to work with in the whole animal setting. I will present a range of delivery technologies we have been investigating including nanopatches, dendrimers, and liposomes. We have developed a fast, reliable and easy method to make siRNA-loaded liposomes for *in vivo* delivery and these particles are highly potent, have low toxicity and have excellent pharmacokinetic properties (Wu et al Pharm Res, 26:512-22, 2009) . We have also investigated ways to improve the efficacy of RNAi and have developed siRNAs and shRNAs that are able to not only silencing target genes but are also able to evoke innate and adaptive immune responses against targeted virally infected cells (Gu et al PNAS, 1 06(20):8314-1 9. 2009) . This new class of "bifunctional" and "trifunctional" siRNAs should be much more effective at treating viral diseases via the induction of Interferon and inflammatory cytokines. Finally, we are currently screening large human siRNA libraries in an effort to identify novel cellular targets whose loss will result in the death of only virally-infected cells. This synthetic-lethal screen will yield a new class of antiviral siRNAs that target cellular genes rather than viral genes.

A number of other technologies including nanopatches, dendrimers and dendrisomes for delivery of siRNA. In terms of efficacy we have been addressing the issue of more potent siRNAs that evoke both innate (bi-functional) and adaptive (tri-functional) immune responses as well as being potent at gene silencing. Our work shows that enhancing immune responses to target genes give excellent results in animal models.

025

INSULIN INCREASES DE NOVO STEROIDOGENESIS IN CASTRATE RESISTANT PROSTATE CANCER-POTENTIAL FOR NEW THERAPEUTIC APPROACHES

C. C. Nelson[1], A. A. Lubik[1], J. A. Locke[2], H. H. Adomat[2], J. H. Gunter[1], E. S. Guns[2], I. McKenzie[1], M. Pollak[3], A. Herington[1], M. Gleave[2]

[1]Australian Prostate Cancer Research Centre, Queensland University of Technology, Brisbane, Australia
[2]The Prostate Centre at Vancouver General Hospital, University of British Columbi, Vancouver, BC, Canada
[3]Departments of Medicine and Oncology, McGill University, Montreal, QC, Canada

Androgen-dependant gene pathways regulate growth and maintenance of both normal and malignant prostate tissue. Androgen-deprivation therapy (ADT), which suppresses androgen production from the testes, exploits this dependence and is frequently used in the treatment of recurrent and metastatic prostate cancer resulting in regression of the tumor. In most cases the regression initially seen with ADT eventually gives way to growth of a population of cancerous cells that no longer require testicular androgens and is referred to as castrate resistant prostate cancer (CRPC) which currently lacks curable treatments. While ADT provides survival benefits, it is associated with a pattern of metabolic alterations consistent with the metabolic syndrome including elevated circulating insulin. We have previously shown that during progression to CRPC prostate tumor cells are capable of synthesizing androgens *de novo.* We have hypothesized that insulin may also help drive CRPC progression; therefore, we specifically examined the effect of insulin on steroid synthesis in prostate cancer cells. We have demonstrated that, in LNCaP prostate cancer cells, insulin increases expression of steroidogenesis enzymes at both the mRNA and protein levels, as well as upregulating the insulin receptor substrate IRS2. Insulin increases PSA secretion, as well as increasing intracellular accumulation and secretion of steroids and androgens. In a mouse xenograft model, tumor progression was associated with increased expression of IRS2 and the insulin receptor. This evidence suggests that elevated insulin accompanying metabolic syndrome, may exacerbate progression to castrate resistance by enhancing steroidogenesis. Integrated therapeutic targeting of these pathways should be explored.

026

ENDOCRINE AND METABOLIC CONSEQUENCES OF WEIGHT LOSS AND OF DIFFERENT WEIGHT LOSS APPROACHES

P. Clifton

Heart and Diabetes Institute, Baker IDI, SA, Australia

Obesity is associated with normal thyroid function, although there are some reports of elevated TSH and T3 which is normalised with weight loss. Some commentators suggest this is an adaptive response to partially restrain fat increases. Weight loss reverses these abnormalities but rapid weight loss induces a sick euthyroid condition with reduced TSH and T3 and reduced resting energy expenditure (REE). High protein weight loss diets in some studies blunt the effects on REE and thyroid hormones. Leptin appears to be linked to TSH release and low dose leptin administration returns energy expenditure, skeletal muscle work efficiency, sympathetic nervous system tone, and circulating concentrations of T4 and T3 to pre-weight-loss levels.

Obesity is also associated with low GH levels with normal levels of IGF-1. GH-binding protein levels are increased and the GH response to GHRH is decreased. These changes are reversed by drastic weight reduction. People with abdominal obesity have an increase in urinary free cortisol but exhibit normal or decreased serum cortisol and normal ACTH levels with an increase in cortisol clearance. There is also an increased response to CRH. Treatment of obesity with very low calorie diets causes a decrease in serum cortisol explained by a decrease in cortisol-binding proteins. The increase in cortisol secretion seen in patients with abdominal obesity may contribute to insulin resistance. Weight loss also lowers renin and aldosterone levels and may explain part of the fall in blood pressure seen with energy restriction.

The metabolic changes seen with energy restriction include large falls in fasting insulin and glucose (if abnormal) and triglyceride. High carbohydrate weight loss diets tend to have minimal falls in triglyceride but do not alter the fall in insulin or glucose.

027

NEW FRONTIERS IN ENERGY EXPENDITURE – THE ROLE OF BROWN ADIPOSE TISSUE

B. Oldfield

Monash University, Clayton, VIC, Australia

Recent insights into the presence and functionality of brown adipose tissue (BAT) in humans have debunked the long held view that BAT is not important in the mediation of energy expenditure in adult humans. This has brought into focus the need to evaluate the role of this tissue in shifts in body weight attributed to drug treatments, different feeding regimes or pathological conditions.

As a precursor to such studies in humans, we have evaluated in rodents the impact of BAT thermogenesis on body weight by modifying the activity of putative central peptidergic mediators of BAT activity and introducing pharmacological treatments known to either reduce or increase body weight. These data are generated in rats using combinations of approaches that involve inhibition of candidate peptides in the CNS and direct measurements of BAT activity in conscious freely moving animals.

028

THE METABOLIC EFFECTS OF ANDROGENS ON ENERGY BALANCE AND VOLUNTARY ACTIVITY

J. Zajac[1,2], K. Rana[1], E. J. Hamilton[1,2], M. Grossmann[1,2], H. E. MacLean[1,2]

[1]Department of Medicine, University of Melbourne, VIC, Australia
[2]Endocrine Unit, Austin Health, VIC, Australia

Androgens have measurable effects on adipose tissue, muscle mass, physical activity and metabolic pathways involved in energy metabolism in both humans and mouse models. In men, withdrawal of androgens in the treatment of prostate cancer results in an increase of total fat mass (3.9kg), visceral fat mass (22%) but a decrease in lean body mass (-1.9kg)

In mouse models in which the androgen receptor (AR) function is ablated, there is an increase in adipose tissue (a very marked increase in some models). We have constructed global AR knockout (ARKO) male mice, which have an in-frame deletion of exon 3 of the AR. These animals have increased subcutaneous (75%) and visceral (36%) fat mass compared to wildtype males. Increased fat mass in global ARKO males is not due to decreased resting energy expenditure or changes in fat oxidation or glucose oxidation rates. ARKO males show reduced voluntary physical activity (70%) and decreased food intake (12%) compared to wildtype. Muscle-specific ARKO males have normal voluntary activity levels indicating that the decreased voluntary activity in global ARKO males is due to AR actions in tissue other than muscle. The logical anatomical location is the brain.

There are a number of questions which need to be addressed:

1. How do androgens regulate fat mass?
2. How do androgens regulate muscle mass?
3. How do androgens regulate food intake?
4. How do androgens regulate voluntary activity?

(1). There is an increase in insulin resistance. Whether this change in energy storage is a result of increased energy intake or decreased energy expenditure is not yet clear.

(1) Androgen deprivation therapy for prostate cancer increases visceral fat and insulin resistance, E.J. Hamilton, E.J. Gianatti, B.J. Strauss, J. Wentworth, D. Lim Joon, D. Bolton, J.D. Zajac, M. Grossma

029

WEIGHT, WEIGHT LOSS & INSULIN RESISTANCE

I. Caterson

Boden Institute of Obesity, Nutrition & Exercise, University of Sydney, Sydney, NSW, Australia

Obesity leads to insulin resistance through various mechanisms and this underlies the development much of the type 2 diabetes mellitus (T2DM) in the world. Obesity is common and its prevalence is increasing worldwide, though there are perhaps indications that the increase is levelling off in some sub-groups. There is no clear relationship between the body's adiposity and insulin resistance, though there is a stronger relationship between the area of visceral adiposity and insulin resistance. In fact, in Japan, it is necessary to have a visceral fat area of >100cm2 to have "obesity disease". This area correlates well with increasing disease and risk. In Australia in those who are obese there is a far greater incidence of T2DM. The concept of metabolic flexibility is another way of describing insulin resistance.

The cause of insulin resistance is still debated after many years of research and investigation. It may have a genetic basis (though not a single gene mutation), it may be due to increased insulin secretion itself in obesity, as demonstrated recently by James et al., or it may be due to presence of intra-tissue lipid (ectopic fat) and higher circulating levels of free fatty acids.

It can be reduced by diet alone. A low fat diet over a 6 week period will almost normalise insulin resistance in those who are obese. However there needs to be ways of reducing insulin resistance and the incidence of diabetes over a greater period. The several diabetes prevention trials show that a small weight loss can achieve this and the effect lasts several years. In trials with obesity pharmacotherapy, there is both improvement in insulin resistance and diabetes control. The far greater weight loss achieved with bariatric surgery may reverse diabetes, particularly in the first few years after its onset.

There is an intimate relationship between obesity, insulin resistance and obesity which diet and or weight loss can alter.

030

WHY DOES ACTIVITY OF THE HPA AXIS OSCILLATE? THE IMPORTANCE OF CONTINUOUS DYNAMIC EQUILIBRATION

S. L. Lightman

Laboratories for Integrative Neuroscience and Endocrinology (LINE), University of Bristol, Bristol, Great Britain

Although HPA axis activity is classically considered to vary over the 24 hours with a single circadian peak and trough, this only represents mean levels of ACTH and cortisol/corticosterone. At the level of the individual human or rodent, the plasma levels of both ACTH and glucocorticoid actually show an underlying ultradian rhythm with maximal size of peaks and troughs at the circadian peak of secretion. These pulses do not appear to correlate with changes in portal CRH secretion and I shall provide evidence that they occur as a result of the feedforward and feedback relationship of the pituitary gland and adrenal cortex, with a fixed delay in the ACTH activation of glucocorticoid secretion and a non-linear feedback inhibition of glucocorticoids on ACTH secretion.

The pattern of the ultradian rhythm of glucocorticoids is very plastic. It can be altered by ageing, sex hormones, lactation and inflammation. It shows genetic variability and can also be programmed by neonatal inflammation and changes in neonatal sex hormone levels.

At the level of extracellular fluid, microdialysis studies in the brain have shown that each pulse of glucocorticoid in the blood is faithfully followed by a pulse of free hormone. Furthermore, both in the hippocampus and in the liver of the rat, we have been able to show that every pulse of corticosterone results in binding of GR to GREs of glucocorticoid responsive genes. This also results in gene pulsing with episodic production of hnRNA. Finally I shall present data that at the systems level, oscillations in glucocorticoid hormone levels are critical for optimal stress responsivity and even behavioural responses.

In answer to the question, why does activity of the HPA axis oscillate, I should like to suggest that the whole axis is in a state of continuous dynamic equilibration that provides optimal responsiveness to changes in the internal or external environment.

031

PEPTIDE ENRICHMENT AND QUANTITATIVE PROFILING TO FACILITATE MARKER DISCOVERY IN PLASMA

V. Wasinger

Bioanalytical Mass Spectrometry Facility, University of New South Wales, NSW, Australia

Plasma is one of the most challenging and complex of the human proteome to use for marker discovery, yet it holds incredible clinical and diagnostic potential for the study of many diseases. Its relatively non-invasive obtainment, presence of potentially detectable levels of all other human sub-proteomes, and advances in technology to work with the large range of proteins present has seen efforts in plasma marker studies continue.

Described here is the development and application of a variety of separation techniques for proteomic and metabolomic research. Pooled plasma was enriched for low mass proteins, peptides and naturally occurring fragments < 25kDa, in addition to the higher mass proteome. We have been able to pinpoint changes occurring at the peptide level in a number of disease states compared to healthy control plasma. Tandem LC-MS using an LTQ-FT Ultra mass spectrometer and quantitation using a label-free approach was used to asses relative abundance changes. Mutliple Reaction Monitoring (MRM) using 4000 Q-trap has been used to follow these relative abundance changes within individual plasma samples.

032

THE ORIGIN OF ANEUPLOIDY IN HUMANS: WHERE WE'VE BEEN, WHERE WE'RE GOING

T. Hassold

Center for Reproductive Biology, Washington State University, Pullman, WA, United States

With the advent of the human genome project in the 1990s, DNA markers became available, allowing us to determine the parent and meiotic stage of origin of human aneuploid conditions. This approach has been extensively used to study the origin of trisomies, with one over-arching conclusion: the vast majority of trisomies derive from errors in the development of the egg and, in particular, from nondisjunction occurring in the first maternal meiotic division (MI). However, against this general background, it has also become apparent that there is considerable chromosome-to-chromosome variation. For example, certain aneuploidies (e.g., the XXY condition, or Klinefelter syndrome) are commonly paternal in origin, while others (e.g., trisomy 16, the most common human trisomy) almost always originate from maternal MI errors. Thus, we now know that individual chromosomes behave differently with respect to mechanisms of meiotic nondisjunction. In this presentation, we will summarize results of these parental origin studies of aneuploidy and discuss the involvement of the first identified molecular correlate of nondisjunction – aberrant meiotic recombination – in the genesis of these abnormalities. We will also look forward, discussing the development of mouse models of human aneuploidy, and recent advances in molecular cytogenetics that make it possible to directly analyze human meiosis "as it happens" in fetal oocytes and in spermatocytes

033

CDH1: A CELL CYCLE PROTEIN INVOLVED IN FEMALE MEIOSIS AND PREVENTION OF ANEUPLOIDY

K. T. Jones

School of Biomedical Sciences, University of Newcastle, Callaghan, NSW, Australia

Mammalian oocytes are arrested at the dictyate stage of prophase I in the ovary. In growing follicles, oocytes can become responsive to Luteinising Hormone and will undergo meiotic resumption just before ovulation. During the first meiotic division, homologous chromosomes are segregated, a process that is very error prone in human oocytes. By ovulation the oocyte has extruded its first polar body and has re-arrested at metaphase of the first meiotic division. Recent work from our lab has established that the protein Cdh1 is involved uniquely in both in the process of prophase I arrest and the correct segregation of homologs in meiosis I. Thus in cultured oocytes, in vitro

antisense knockdown of Cdh1 induces both meiotic resumption and high rates of aneuploidy as a result of non-disjunction during first meiosis. Cdh1 causes prophase I arrest by inducing cyclin B1 degradation and maintaining low levels of the kinase CDK1, whose activity induces meiotic resumption. Cdh1 is an activator of the Anaphase-Promoting Complex (APC), a ubiquitin ligase that earmarks proteins such as cyclin B1 for proteolysis. Cdh1 prevents aneuploidy by causing the degradation of Cdc20, a protein that is responsible for activating the APC once all homologs are correctly aligned at metaphase. Thus loss of Cdh1 seems to prematurely activate APC(Cdc20) activity. It is interesting that a single protein can affect two important meiotic transitions in oocytes. However to explore its functions more fully, and confirm that an in vitro knockdown is faithfully replicated by in vivo loss, a targeted knockout of Cdh1 is needed. Therefore we have generated an oocyte specific Cdh1 knockout by ZP3 promoter driven Cre- recombinase activity in oocytes carrying loxP insertions in the single copy Cdh1 gene. This talk will therefore focus on the effects of an in vivo Cdh1 knockout.

034

IMPORTIN A2 MEDIATES SUBNUCLEAR TARGETING OF THE CAJAL BODY COMPONENT COILIN; A KEY ROLE IN SPERMATOGENESIS?

J. D. Ly-Huynh[1,2], A. Efthymiadis[2], P. Whiley[1,3], E. Richards[3], H. Lescesen[1,2], I. Meier[4], G. A. Matera[4], K. L. Loveland[1,3], D. A. Jans[1,2]

[1]ARC Centre of Excellence in Biotechnology and Development, Australia
[2]Department of Biochemistry and Molecular Biology, Nuclear Signalling Lab, Monash University, Melbourne, VIC, Australia
[3]Departments of Biochemistry and Molecular Biology/Anatomy and Developmental Biol, Monash University, Melbourne, VIC, Australia
[4]Departments of Biology/Genetics, University of North Carolina, Chapel Hill, United States

Spermatogenesis, the progressive maturation of immature germ cells to form spermatozoa, requires nucleocytoplasmic shuttling of nuclear factors to implement changes in gene transcription, as well as the storage and alternative splicing of mRNA transcripts in the nucleus that is vital for fertility. The key cellular mediators of nuclear entry are members of the importin (IMP) superfamily, of which the five different IMPα proteins in mouse testis are expressed dynamically throughout spermatogenesis, consistent with roles in transporting distinct, specific cargoes critical to gamete maturation. We identified the central Cajal body (CB) component Coilin as a specific testicular binding partner of IMPα2 in a yeast 2-hybrid screen and confirmed this interaction by coimmunoprecipitation from testis lysates. CBs are small nuclear inclusions that can associate with histone genes and facilitate histone pre-mRNA processing by recruiting RNA processing factors; intriguingly, expression of IMPα2 but not other IMPαs can regulate the number and size of CBs, as shown in cell culture experiments. Immunohistochemical analysis revealed that Coilin is predominantly in spermatocytes and in round and elongating spermatids in the rodent testis. The physiological importance of its role is indicated by the fact that Coilin knockout mice have reduced fertility, smaller testes and aberrant spermatogenesis. Our future work will focus on the testicular functions of Coilin and IMPα2 during spermatogenesis, and their roles in coordinating the pre-assembly, storage and targeting of transcription complexes to RNA processing machinery in the nucleus.

036

CAREER PATHWAYS IN CLINICAL RESEARCH

L. A. Bach

Endocrinology and Diabetes, The Alfred, Melbourne, VIC, Australia

Research is an exciting and worthwhile component of a medical career. Trying to work out how to make a start in research may appear daunting, but answering a few key questions can get you on your way: Am I interested in basic or clinical research? What area of endocrinology fascinates me? Am I interested in a full-time academic career? What do I hope to achieve by undertaking a period of research? For many trainees, the next step is to enrol in a higher degree. You need to find an appropriate supervisor, so ask your colleagues and mentors for advice. Speak both to potential supervisors AND their students. There is a range of scholarships available; your university's research office will help you identify these. If you are bitten by the research bug, the next step is to extend your experience. This may be an opportunity to travel overseas, which I strongly recommend. Again, there are funding opportunities to help you with this step in your career development. By this point, you will need to ask yourself some of the same questions as those listed above. If you are serious about a full-time academic career, options include working at a university, research institute or a teaching hospital. Having your own funding increases your flexibility, so you may want to apply for career development awards and/or practitioner fellowships. It is possible to continue to participate in research even if you decide not to pursue it as a full-time career. Many public hospital departments conduct clinical trials, and becoming part of a research team is a viable option. Clinical research in private practice or a private hospital is also an emerging possibility. Apart from the pursuit of knowledge, research participation enhances our clinical thinking, improves our teaching, and is a lot of fun.

037

GETTING YOUR FIRST PAPER PUBLISHED

J. Ho

Endocrinology, Flinders Medical Centre, Bedford Park, SA, Australia

Scientific writings represent final products of scientific endeavours. Many individuals find it difficult to write informatively about their clinical practice or to translate the outputs of academic studies into accessible publications.

The objective of this session is to discuss the reasons that manuscripts fail to be published and to establish some principles for increasing the likelihood of publication. The discussion concludes by emphasizing that success requires undertaking the necessary preparatory work, time, commitment and enthusiasm.

038

ESTABLISHING AN INDEPENDENT RESEARCH CAREER

M. K. O'Bryan

Dept. of Anatomy and Developmental Biology, Monash University, VIC, Australia

Making the transition from a successful post-doctoral researcher to heading your own lab is an exciting but potentially challenging time within the Australian research environment. Critically this transition depends upon research outputs (including publications, grants, awards) and the perception of independence. Researchers need to accept that a career in research is something that needs to be planned for over the long term and that the strategic answer to a particular question may change depending on your stage of career. Researchers need to have a clear picture of their KPI eg. related to teaching, research, professional service, and they need to make an honest assessment of what they are willing to do in order to achieve research success. Success can be positively influenced by aligning yourself with a well established research group with complementary (but distinctly different skills), the establishment of research networks which are likely to lead to co-authorships and joint grants, having a mentor to filter scientific and strategic ideas through, and having open and honest conversations with previous employers. Critically, researchers need to be aware of how funding agencies read and score research output. Points to consider may include: the number and caliber of papers you publish, the relative percentage of primary and middle authorships, are you publishing manuscripts every year, the attainment of research-based awards (even small ones) and grants, investment from your home institution, and speaking invitations both nationally and internationally. Ultimately like many careers, success in research is related to natural ability, being organized, being pro-active, planning and working hard. While there will no doubt be bumps along the way, I believe this is an achievable and highly rewarding path.

039

MAKING A COMPETITIVE CV

G. Risbridger

Prostate & Breast Cancer Research Program Faculty of Medicine, Nursing and Health Services Monash University, VIC, Australia

Generating, and maintaining, a CV is an ongoing task that begins as one embarks upon an academic career; making it competitive is necessary at all stages of career development and progression. The first (and subsequent) application that requires a CV will require precision and clarity of presentation of your records of achievement; therefore make sure the records of duties, accomplishments and excellence are all recorded systematically for retrieval. After the first few applications, feedback on elements of one's experience will become available and at each time, note where the strengths and weaknesses are perceived/exist and develop strategies to address them.

Specific career paths and choices will determine some key features of a more competitive CV, since many selection criteria are obvious. Making a CV more competitive, requires one to acknowledge and identify those key elements as personal goals and ambitions are set and evaluation of what is needed to ensure they become components of a CV. Perhaps some will require short-term strategies, but others may take time and opportunity for which assistance from colleagues or mentors will be necessary and should be sought.

CVs come in different forms: full (40-50 pages), short (4-6 pages) and brief (a paragraph). Each of them takes a discipline to maintain in competitive form, but once completed, a CV is a very personal document. Not only should it reflect your current status and achievements, but it should also capture your future goals and the notion that you have yet to achieve your full potential.

041

ARE ENVIRONMENTAL EXPOSURES AFFECTING HUMAN REPRODUCTIVE HEALTH?

P. Hunt

Center for Reproductive Biology, Washington State University, Pullman, WA, United States

The concern that human reproductive health may be affected by chemicals in our daily environment has grown in recent years with the recognition that; 1) some countries have seen a recognizable decline in sperm counts and an increase in urogenital tract abnormalities among newborn males, 2) the incidence of some cancers has increased precipitously, and 3) the number of infertile couples has increased markedly in many countries. Our laboratory focuses on the oocyte and the factors that cause the production of chromosomally abnormal eggs. We know that the risk of a chromosomally abnormal pregnancy is strongly influenced by maternal age, but there is growing concern that environmental exposures may influence the ability of both the male and female to produce normal gametes. Our laboratory has focused on the effect of exposures to a ubiquitous chemical to which humans are exposed daily, bisphenol A (BPA). BPA is used in a wide variety of consumer products from plastics and resin coatings to eyeglasses and pressure printed receipts. Our studies in mice demonstrate that BPA exposure during fetal development adversely affects female fertility because BPA influences several significant stages of egg development. In the male mouse, we and others have found that prenatal, perinatal, and adult exposures can affect the function of the testis. In current studies we are attempting to determine if effects seen in the mouse are also a feature of BPA exposed primates. We are using a rhesus monkey model to determine how BPA is metabolized in the pregnant and nonpregnant female and how BPA exposure influences the developing fetus. Lastly, in human studies we are evaluating BPA levels in the developing fetus and assessing their effect on the developing fetal ovary.

042

ENDOCRINE DISRUPTING IMPACTS IN RECEIVING WATERS OF THE SYDNEY BASIN

R. Lim

Department of Environmental Sciences, University of Technology Sydney, Ultimo, NSW, Australia

Water reuse for a number of activities including potable water and replacement of environmental flows is becoming more significant due to the prolonged drought Australia has recently experienced. There is also much debate regarding potential impacts of compounds such as steroid endocrine disrupting chemicals (EDCs), pharmaceuticals & personal care products (PPCPs), and persistent organic pollutants (POPs) to environmental and human health. This paper presents an overview of findings on some EDCs in the Sydney Basin to assess the environmental risk they pose.

A tiered approach, using a suite of endpoints spanning *in vitro* (e.g., estrogen receptor binding assay, the 2-hybrid yeast test) to *in vivo* (using the mosquitofish (*Gambusia holbrooki*) to assess vitellogenin induction, and morphological and behavioural changes) studies was conducted on aquatic systems receiving urban and treated sewage effluents. *In vitro* bioassays suggest low levels of estrogenicity in sewage contaminated waterways. Both estradiol (E2) and estrone (E1) were identified in all river water samples, suggesting that sewage contamination is widespread. The synthetic hormone, ethynylestradiol (EE2), was below detection limits in all samples tested. Results indicate that the STPs were not the only source of EDCs in aquatic systems within the Sydney area. Improvements in treatment technologies in STPs have substantially reduced EDC levels in final effluent as indicated by a reduction in endocrine disrupting effects on the mosquitofish over several years of study. In addition, advanced tertiary treatment technology removed EDCs to levels below that measurable by *in vitro* assays and *in vivo* fish testing. This tiered weight of evidence approach provided insights to the risks EDCs in sewage effluent produced from current treatment technologies have on the environment.

043

THE EFFECT OF THE INSECTICIDE PYRIPROXYFEN ON OVARY SYNTHESIS IN THE CHRISTMAS ISLAND RED CRAB, *GECARCOIDEA NATALIS*; A POSSIBLE CASE OF ENDOCRINE DISRUPTION?

S. M. Linton[1], L. Barrow[2], C. Davies[3], L. Harman[1]

[1]School of Life and Environmental Sciences, Deakin University, VIC, Australia
[2]King Island Natural Resource Management Group, King Island, TAS, Australia
[3]Marine and Atmospheric Research, CSIRO, Brisbane, QLD, Australia

The yellow crazy ant, *Anopholepis gracilipes* is an invasive species on Christmas Island, Indian Ocean whose population needs to be controlled before there is irrevocable environmental damage. The insecticide, pyriproxyfen, a compound which mimics juvenile hormone of insects, has been proposed to do this. Before it can be used in the field, its effects on non target species such as the endemic red crab, *Gecarcoidea natalis*, need to be investigated. Land crabs, like all decapods, may utilise a similar hormone called methyl farnesoate which is thought to be involved in controlling early ovary development. Pyriproxyfen may also mimic methyl farnesoate and thus disrupt this process. The effect of pyriproxyfen on early ovary synthesis in *G. natalis* was investigated by feeding crabs a mixture of leaf litter and bait containing 0.5% pyriproxyfen (experimental groups) or a mixture of leaf litter and bait containing no pyriproxyfen (control groups) at simulated baiting doses (2 kg ha^{-1} and 4 kg ha^{-1}). Two additional groups of crabs were fed *ad libitum*, either bait containing 0.5% pyriproxyfen or the control bait. Experiments were conducted from July to September of 2007. Red crabs synthesise their ovaries annually over two months (July to September) in the dry season. This situation of high nitrogen demand is funded from small excesses of nitrogen assimilated from a mainly leaf litter diet. Pyriproxyfen affected early ovary development. Ovaries from crabs in the experimental groups at all baiting levels had a higher total nitrogen content and dry mass than that of the control animals. The ovaries from the experimental

animals were also more mature; they contained more previtellogenic and early vitellogenic oocytes, of a larger diameter, than ovaries of the control animals. Significant amounts of pyriproxyfen were accumulated in the target tissues, the midgut gland and ovary. Minor amounts of pyriproxyfen were accumulated in muscle, a non-target tissue. By mimicking methyl farnesoate, pyriproxyfen may have caused endocrine disruption in *G. natalis*. In particular it may have stimulated early ovary development and synthesis of yolk protein.

044

PREVENTION AND TREATMENT OF TYPE 2 DIABETES: A SOUND APPROACH BASED UPON ITS PATHOPHYSIOLOGY

R. De Fronzo

University of Texas Health Science Center, Texas, United States

Insulin resistance in muscle and liver and progressive b -cell failure represent the core pathophysiologic defects in type 2 diabetes mellitus (T2DM). The b -cell failure occurs long before the onset of overt diabetes and is more severe than previously appreciated. Subjects in the upper tertile of impaired glucose tolerance (IGT) are maximally/near-maximally insulin resistant and have lost approximately 80% of their b -cell function. In addition to the muscle, liver and b-cell (triumvirate), the fat cell (accelerated lipolysis), gastrointestinal tract (incretin deficiency/resistance), a -cell (hyperglucagonemia), kidney (increased glucose reabsorption), and brain (insulin resistance) all play important roles in the development of glucose intolerance in type 2 diabetic individuals. Collectively, these eight players comprise the ominous octet and dictate that: 1) multiple drugs used in combination will be required to correct the multiple pathophsyiological defects, 2) treatment should be based upon reversal of known pathogenic abnormalities and not simply on reducing the A1c, and 3) therapy must be started early at the stage of IGT/IFG to prevent/slow the progressive b -cell failure that already is well established. In IGT subjects pharmacologic therapy with thiazolidinediones (TRIPOD, PIPOD, DREAM, ACT NOW), metformin (US DPP, Indian DPP), and combined TZD-metformin (CANOE) therapy have been shown to be very effective in reducing the conversion rate of IGT to T2DM. For type 2 diabetic patients, a treatment paradigm shift is recommended in which combination therapy is initiated with diet/exercise, metformin (which improves insulin sensitivity and has antiatherogenic effects), a thiazolidinedione (TZD) (which improves insulin sensitivity, preserves b -cell function, and exerts antiatherogenic effects), and exenatide (which preserves b -cell function and promotes weight loss).

045

TESTOSTERONE, BODY COMPOSITION AND THE AGEING MALE

C. Allan

Prince Henry's Institute, VIC, Australia

Male ageing is associated with a modest decline in serum testosterone and adverse body composition changes, specifically a reduction in skeletal muscle and an increase in body fat. Testosterone supplementation has been shown to partially reverse these changes but its role in the ageing male remains controversial. It is now increasingly recognized that a bi-directional relationship exists between obesity and testosterone. Obese older men experience a greater age-related decline in serum testosterone than their non-obese peers and are more likely to have documented biochemical hypoandrogenism. Conversely, testosterone therapy is able to reduce total body fat with a greater effect seen in men with higher baseline fat mass. There are limited data examining the effect of exogenous testosterone on regional fat mass, in particular, visceral adiposity. Our studies have shown that, in non-obese older men with low-normal testosterone levels, 12-months of testosterone therapy is able to prevent gain in visceral fat when compared to placebo. We have recently completed a 12-month randomized controlled trial of intramuscular testosterone therapy, in addition to healthy lifestyle advice, in obese middle-aged and older men. Testosterone treatment reduced total body fat by 10% and increased skeletal muscle mass by 6%. Subcutaneous abdominal fat decreased by 9%. Larger, long-term studies are now needed to understand the role of exogenous testosterone in modifying body composition and subsequently cardio-metabolic risk in the ageing male.

046

MECHANISMS OF OBESITY IN THE OFFSPRING OF DIABETIC PREGNANCY

S. M. Lau[1,2,3], S. Lin[1], R. Stokes[1], K. Cheng[1], K. M. Cha[1], P. F. Baldock[1], R. F. Enriquez[1], M. McLean[2,4], N. W. Chrung[2], A. Sainsbury[1], H. Herzog[1], J. E. Gunton[1,2]

[1]Garvan Institute of Medical Research, Darlinghurst, Sydney, NSW, Australia
[2]Diabetes and Endocrinology, Westmead Hospital, Randwick Sydney, NSW, Australia
[3]Diabetes and Endocrinology, Prince of Wales Hospital, Randwick Sydney, NSW, Australia
[4]Clinical School, University of Western Sydney, Sydney, NSW, Australia

The origins of obesity lie in the complex interaction between genes, diet/lifestyle and the early developmental-environment. Diabetes in pregnancy (DP) is linked to obesity in the offspring but the mechanisms are not fully understood, and debate persists regarding the maternal factors and nutrients responsible. We used a novel model to examine effects of DP on offspring. Research design and methods: ß-cell-specific Aryl-hydrocarbon Receptor Nuclear Translocator-null (ß-ARNT) mice develop mild DP from ß-cell dysfunction. ßARNT and

floxed-control females were used to obtain DP and non-DP (NDP) respectively. Results: DP offspring became spontaneously obese on a normal chow diet. They were heavier than NDP offspring, with increased body fat. Respiratory exchange ratio (RER) was higher in DP offspring, indicating decreased capacity to switch to lipid oxidation. Metabolic rate was decreased prior to onset of obesity. Increased weight was more pronounced in ßARNT DP offspring, which had increased hypothalamic neuropeptide Y (NPY), and decreased POMC expression. Weight, body fat, insulin sensitivity and RER in all mice, and NPY in ßARNT mice, were significantly correlated with maternal pregnancy glucose tolerance ($p<0.01$) but not with litter size or maternal weight, triglycerides or pre-pregnancy glycaemia. Conclusions: Offspring from DP developed spontaneous obesity with increased fat and RER. There was an interesting interaction between in utero exposure and offspring genotype, with ßARNT DP offspring exhibiting a more severe phenotype than floxed-control DP offspring. Maternal glucose was the primary factor 'programming' the phenotype.

047

ANDROGEN RECEPTOR SIGNALLING IN HUMAN BREAST TISSUES AND ITS IMPLICATIONS FOR BREAST CANCER

T. Hickey

Breast Cancer Research Group, Hanson Institute, SA, Australia

In the past decade, androgen receptor (AR) signalling has emerged as a major regulatory pathway in breast cancer, with androgenic effects dependent upon the molecular phenotype of the tumour. The most common phenotype is luminal (estrogen sensitive) breast cancer, and in this type of disease AR signalling appears to play a protective role. In contrast, AR signalling may promote growth of a subset of estrogen insensitive breast cancers, highlighting the need to better define AR signalling in normal and malignant breast tissues. Our laboratory has shown that AR signalling inhibits estrogen receptor alpha (ER a) signalling in ER a + breast cancer cell lines and that a low level of AR protein is associated with poor outcomes in women with ER a + disease. Our current work focuses on defining AR signalling characteristics in the normal human breast epithelium and determining whether compromise of this signalling pathway may predispose to development of estrogen sensitive breast cancer. We find that AR+ epithelial cells always exceed ER a + epithelial cells in normal breast tissues from women without breast cancer and that menopause is associated with a reduction in the average AR:ER a ratio (13:1 in pre-menopausal versus 6:1 in post-menopausal women; $p<0.05$). Cancer in the breast is also associated with a significant reduction in the AR:ER a ratio in the normal breast epithelium excised distal to the tumour in pre- and post-menopausal women. This reduction is most striking in post-menopausal women with breast cancer who possess an average AR:ER a ratio of 2:1, largely due to increased levels of ER a , not a reduction in levels of AR. Interestingly, cultured normal breast tissues from post-menopausal women with breast cancer have abnormal AR signalling characteristics. When in situ or invasive ER a + breast tumour tissues are compared to normal epithelium from the same breast, AR and ER a levels are both significantly increased, but the AR:ER a ratios are decreased to 1:1 or less and PSA, a marker of functional AR activity, is dramatically reduced. Collectively, these data provide evidence to support the concept that perturbation of AR signalling is evident in luminal breast cancers and may occur early in the disease process.

048

THE ROLE OF TNFA IN OESTROGEN BIOSYNTHESIS AND BREAST CANCER

S. Q. To[1,2], K. C. Knower[1], E. R. Simpson[1,2], C. D. Clyne[1,2]

[1]Prince Henry's Institute, Clayton, VIC, Australia

[2]Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia

Breast cancer is the leading cause of cancer-related death amongst women in the western world. In post menopausal women, approximately 70% of breast tumours can be classified as Oestrogen Receptor positive (ER+), and oestrogens are strongly associated with continuing development and progression of such cases. Adjuvant anti-oestrogen therapies are pivotal in the treatment of ER+ cancers, and work is ongoing to improve the effectiveness of this approach.

In post-menopausal women, adipose tissue is the primary site for oestrogen biosynthesis. Local levels of the hormone in a breast tumour micro-environment are as much as 10 times greater than in the circulation of post-menopausal women. This appears to be due to the enzyme responsible for conversions for conversion of androgens to estrogens, aromatase, is markedly upregulated within a tumour and its surrounding environment. This upregulation is seen as a result of a production of factors by the tumour to stimulate aromatase activity and thus maintain a constant source of oestrogens.

Tumour Necrosis Factor α (TNFα) is one such factor known to stimulate expression of aromatase in breast adipose fibroblasts (BAFs). It does so by turning on the gene encoding aromatase, CYP19A1, via the tissue-specific promoter I.4 (PI.4) and thus regulating oestrogen biosynthesis in these cells. Numerous studies have correlated increased levels of TNFα to hallmarks of breast-cancer risk, most notably increased weight and advanced age. Whilst it is known that TNFα stimulates CYP19A1 via PI.4, the precise mechanisms by which this occurs remains elusive. Our work aims to pinpoint signalling pathways and transcription factors by which TNFα is working to regulate oestrogen biosynthesis, so that this information may be harnessed in the development of novel diagnostic markers and therapeutics in the continued fight against breast cancer.

049

RNA-BINDING COFACTORS IN NUCLEAR RECEPTOR-MEDIATED GENE REGULATION

P. J. Leedman[1,2], S. M. Colley[1], K. Iyer[1,2], A. Redfern[1,2], M. Epis[1], D. J. Beveridge[1], L. M. Stuart[1], K. Ramsay[1], V. Russell[1], E. Hatchell[1,2], A. Gatignol[3], R. Lanz[4], C. Foulds[4], B. W. O'Malley[4]

[1]Laboratory for Cancer Medicine, Western Australian Institute for Medical Research, Perth, WA, Australia
[2]School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia
[3]Virus-cell interactions laboratory, Lady Davis Institute for Medical Research, McGill University, Montreal, Quebec, Canada
[4]Division of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, United States

Nuclear receptor (NR) signaling is modulated by the recruitment of coregulators (coactivators or corepressors), which provide high fidelity control of transcriptional signaling. The coregulator landscape was altered significantly some years ago when SRA, an RNA coregulator, was identified by the O'Malley group[1]. We set out to identify novel SRA-binding coregulators that could serve as potential targets for therapeutics in hormone-dependent cancer. SLIRP, a novel small SRA-binding protein[2], was identified and found to be a potent repressor of NR and Notch signaling in prostate and colorectal cancer. Unexpectedly, SLIRP is predominantly mitochondrial, and also regulates mitochondrial gene expression and apoptosis. Subsequent studies have identified a role for SLIRP in metabolism and in mitochondria-rich tissues. A SLIRP knock-out mouse is providing additional insight into the functional biology of this remarkable small protein. We have also characterized a second group of SRA-binding proteins, which includes PACT and TRBP, components of the multiprotein RNA-induced-silencing (RISC) complex, together with PKR, and shown them to be NR coactivators that regulate NR signaling . Interestingly, Dicer a central component of the RISC complex that processes microRNAs (miRNAs), is also a NR coregulator. SRA facilitates recruitment of these proteins to target promoters and also associates with them in the cytoplasm. In addition, specific pre-miRNAs associate with these members of the RISC in the nucleus in a hormone-dependent manner. These data demonstrate a new functional nuclear repertoire for PKR, PACT, TRBP and Dicer as coactivators of SRA-mediated NR signaling. Furthermore, the association of members of RISC with pre-miRNAs in the nucleus suggests new linkages between NR-mediated transcription and the factors involved in miRNA processing. Taken together, the SRA-binding NR coregulators are providing novel insight into NR signaling, uncovering new functional roles for proteins involved in miRNA processing and identifying new potential therapeutic targets in endocrine signaling.

(1) Lanz et al., (1999) Cell, 97:17-27.
(2) Hatchell et al., (2006) Mol Cell, 22: 657-68.

050

NOVEL MECHANISMS IN GH SIGNALING

M. J. Waters, A. J. Brooks

Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD, Australia

Growth hormone is the major regulator of postnatal growth and final height, as well as a major regulator of fuel supply during fasting. As a result of decades of work we have a clear understanding of the structures of both the hormone and the extracellular domain of its receptor, and their interaction, as well as the signaling processes which result from this interaction. In particular, the key role of STAT5 in driving IGF-1 expression has been established, as well as the means of its activation by the tyrosine kinase JAK2. Recently we and others have shown that the GH receptor exists as a constitutive dimer, and that activation by GH involves a reorientation of the two receptors by the hormone leading to activation of the associated JAK2 and its STAT5 target. Using extensive mutagenesis and FRET studies, together with molecular dynamics, we have data supporting a combined rotation and scissor movement induced by hormone binding. This movement separates the submembrane box 1 sequences which bind the JAK2, and we have a model which explains how this movement could activate the JAK2. Our constitutively active receptor constructs with separation of the box 1 sequences may have applicability in tissue-specific regeneration. We have also created targeted knockin mutations to the GH receptor cytoplasmic domain to identify the signaling pathways involved in the regulation of growth and metabolism. These have supported the importance of STAT5 generation in postnatal growth, and provided microarray data correlating different signaling domains with altered transcript expression. In particular, we have been able to derive a model for the generation of hepatic steatosis which occurs in adult GH deficiency. Finally, we have been able to show that the GH receptor signals not only through JAK2, but as with related class 1 cytokine receptors, also through a Src family kinase to activate ERK signaling. This is needed for hepatic regeneration after partial hepatectomy. Supported by grants from the NHMRC (Australia) to MJW.

051

THE ORPHAN NUCLEAR HORMONE RECEPTOR NR4A SUBGROUP, TARGETS OF BETA-ADRENERGIC SIGNALING: UNDERSTANDING THE ROLE OF NR4A3/NOR-1 IN IN SKELETAL MUSCLE METABOLISM

M. Pearen, R. Fitzsimmons, N. Eriksson, M. Wang, G. E.O. Muscat

Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD, Australia

We have previously provided evidence for crosstalk between the NR4A subgroup of orphan nuclear receptors (Nur77, Nurr1 and Nor-1) and beta adrenergic signaling in skeletal muscle [in vitro and in vivo, Maxwell MA et al. 2005, J. Biol. Chem. 280:12573-; Pearen MA et al 2006 Endocrinology 147, 5217-; Pearen MA et al 2008, Endocrinology 149, 2853-; reviewed in Pearen MA and Muscat GEO Mol. Endo. 2010 Apr 14. (Epub - PMID: 20392876)] . Moreover, beta2-AR agonist treatment markedly and transiently induced expression of the mRNAs encoding the NR4A subgroup in several other major mass metabolic tissues (Myers S et al 2009 Mol. Cell. Endocrinol. 309, 101-).

The dramatic and transient beta-adrenergic mediated induction (within 15-60 min) of the mRNAs encoding these nuclear receptors is concordant with the involvement of protein kinase A, MAPK and phosphorylation of cAMP response element-binding protein. Knockdown of NR4A3/Nor-1 using siRNA in skeletal muscle cells (in vitro) decreased palmitate oxidation and increased lactate accumulation consistent with a shift to anaerobic metabolism. Candidate-based mRNA expression profiling after expression of a Nor-1siRNA (relative to a negative control siRNA) resulted in the significant differential expression of genes that control fatty acid oxidation and utilization of pyruvate (Pearen MA et al 2008, Endocrinology 149, 2853-). These studies indicated Nor-1 expression was necessary for oxidative metabolism. Complete analysis of skeletal muscle gene expression (utilising the Illumina 46K mouse BeadArrays) following (acute and chronic) systemic administration of beta(2)-AR agonists revealed significant differential expression of genes associated with hypertrophy, myoblast differentiation, metabolism, circadian rhythm, transcription, histones and oxidative stress (Pearen MA et al 2009, BMC Genomics 10, 448-). We are producing transgenic mice that over-express NOR-1 gain and loss of function vectors in skeletal muscle [by using the human skeletal alpha actin (HSA) promoter] to investigate the in vivo function of this NR in metabolism. Preliminary analysis of this mouse model, Tg-HSA-VP16-Nor-1, suggests Nor-1 is involved in the regulation of glucose homeostasis.

052

HBA1C – THINGS ARE CHANGING - DIAGNOSTIC ROLE AND NEW REPORTING

P. Colman

Department of Diabetes and Endocrinology, Royal Melbourne Hospital, VIC, Australia

HbA1c is well established as the gold standard marker of glycemic control and, given its ability to reflect time averaged blood glucose during the previous 2-3 months, its possible role as a diagnostic for diabetes has long been discussed. The traditional use of glucose as a diagnostic is hamstrung by the need for fasting tests or an oral glucose tolerance test and there is major day to day variability of glucose. In contrast, HbA1c can be measured at any time of day and does not require any special preparation for testing. The American Diabetes Association has now recommended that HbA1c can be used to diagnose diabetes (1). The diagnosis is made if the HbA1c level is > 6.5%; the ADA suggest that 5.7 to 6.4% is a high risk range and that those with HbA1c between 6.0 and 6.5% are at particularly high risk and might be considered for diabetes prevention. WHO are considering a diagnostic use of HbA1c and are likely to provide a similar recommendation.

What are the issues with use of HbA1c as a diagnostic? HbA1c can be affected by a variety of factors – haemoglobinopathies, anaemia and disorders with increased red cell turnover. In particular, altered red blood cell turnover and haemodilution render it impractical for diagnosing gestational diabetes. Maintaining excellent precision and consistency of measures between labs using different assay methods is also critical. Cost and availability of HbA1c assays will be a problem in developing countries.

The unit of reporting is also a continuing issue with HbA1c assays. Currently in Australia HbA1c is reported as a DCCT related level. Some pathology laboratories are also measuring the estimated average glucose (eAG)(2). Recently, the IFCC have developed pure reference standards which can be used to 're-standardize' the HbA1c assay. When the new IFCC standardization procedure is adopted the HbA1c percentage values will be lowered due to the higher specificity of the reference method. The changeover to different HbA1c units across the world has commenced (3). Australia is yet to confirm a commencement date for reporting of the new unit.

(1) International Expert Committee on the role of the A1c assay in the diagnosis of diabetes. Diabetes Care 32:1327-34, 2009

(2) Nathan DM, Kuenen J, Borg R, Zheng H, Schoenfeld D, Heine RJ. Translating the A1c assay into estimated average glucose values. Diabetes Care 31:1473-8, 2008

(3) Hanas R, John G on behalf of the International HbA1c Consensus committee. 2010 consensus statement on the Worldwide standardization of the haemoglobin A1c measurement. Ann Clin Biochem 47:290-2

053

CHOOSING AND USING THRESHOLDS IN DIABETES DIAGNOSIS AND MANAGEMENT: GESTATIONAL DIABETES

A. McElduff

NSEC, St Leonards, NSW, Australia

The Hyperglycemia and Adverse Pregnancy Outcome (HAPO) study assessed the risks of various adverse fetal and pregnancy outcomes associated with 0, 1, and 2 hour plasma glucose values (less than overt diabetes) in an oGTT performed between weeks 24-32 of gestation. HAPO demonstrated a linear relationship between each of these glucose values and the risk of an adverse outcome for either the fetus or the mother. Thus, the diagnosis of GDM is arbitrary. The current International Association of Diabetes and Pregnancy Study Groups (IADPSG) consensus identified the 0, 1, and 2 hour plasma glucose values that increased the risk of an adverse outcome by 75% over that of mean glucose values. Adverse outcomes included (birthweight >90th percentile, cord c-peptide >90th percentile, and neonatal % body fat >90th percentile). Levels which give a 74% increase are regarded as normal even though when interacting with other risk factors i.e. obesity, the risk of an adverse outcome is greater than 75%.

The IADPSG has recommended that the glucose challenge test be abandoned at least in part because it is not a very good screening test with poor sensitivity and specificity.

Given the previous discussion of the linear relationship between glycaemic levels and pregnancy outcomes and the interaction with other risk factors, it will not surprise that treatment targets or levels for initiating insulin therapy are not clear. In at least one study over vigorous treatment has resulted in an increase in the adverse outcome of small for gestational age infants.

A pragmatic approach, supported by the two recent large RCTs in GDM, will be discussed.

054

OTHER TARGETS IN DIABETES

P. Phillips

The Queen Elizabeth Hospital, SA, Australia

Abstract not available at time of print.

055

TROPHOBLAST, PLACENTA AND EARLY EMBRYO: HOW THE MARSUPIAL DEVELOPS

M. B. Renfree, S. R. Frankenberg, C. Freyer

ARC Centre of Excellence for Kangaroo Genomics, Department of Zoology, The University of Melbourne, VIC, Australia

In marsupials, the blastocyst forms as a single cell layer of cells. The marsupial blastocyst has no inner cell mass, so the 80-100 cell tammar embryo remains in diapause as a unilaminar blastocyst. All marsupials have a unilaminar stage, but what is unusual is that in the tammar the total cessation of cell division and cell metabolism lasts for 11 months each year.

Marsupials are placental mammals. The yolk sac forms the definitive placenta up to birth. Only very few marsupials, such as the bandicoot, have a chorio-allantoic placenta, which supplements the placental functions of the yolk sac. However, the understanding how the unilaminar layer of trophoblast cells of the diapausing blastocyst become specified into placental and embryonic tissues has been an ongoing puzzle. To identify genes that do become differentially expressed in tammar development, we targeted the stage of the earliest appearance of the embryonic disc, at which the remainder of the blastocyst is then defined as trophoblast, as well as early cleavage stages. Intriguingly, we found no evidence for early differential expression of the canonical pluripotency genes *POU5F1*, *SOX2* and *NANOG*, or of *CDX2*. By contrast, we found overt differential expression of *GATA3*, the closely related gene *GATA2*, and *FGF4*. This expression profile suggests that in the tammar, mechanisms regulating trophoblast- and pluriblast-specific expression of *POU5F1*, *SOX2*, *NANOG* and *CDX2* are temporally secondary to those regulating *GATA2* & *-3* and *FGF4* expression. Together, our results may signify the evolution of alternative mechanisms of early lineage specification in marsupials, or alternatively reveal a general hierarchy of signalling mechanisms that are masked in the relatively rapid and "compressed" development of mice. The results of our ongoing study have important implications for understanding not only marsupial stem cells but the early development of all therian mammals.

056

EVEN REPTILES DO IT, THE STRUCTURE AND FUNCTION OF PLACENTAE IN VIVIPAROUS LIZARDS

M. Thompson

School of Biological Sciences, University of Sydney, NSW, Australia

Live birth (viviparity) has evolved independently more than 100 times in squamate reptiles (lizards and snakes). Most viviparous lineages are characterised by a simple placenta with squamous epithelia on both maternal and embryonic sides, and the remnants of an eggshell persist for some, if not all, of pregnancy. The embryos are predominantly lecithotrophic, with maternal-embryonic exchanges being limited mostly to inorganic ions, water and respiratory gases. Nevertheless, there is differentiation of a chorioallantoic placenta and a yolk sac (omphalo) placenta in all species. Complex placentae have evolved in the Squamata in only four or five lineages, all in the lizard family Scincidae (skinks). In species with complex placentation, the uterus differentiates to allow different functions in association with each embryonic membrane type. Species with complex placentae are characterised by a hypertrophy of maternal and embryonic cells, elaboration of the maternal surface, a reduction in yolk with a concomitant increase in placentotrophy and, in some, regional differentiation of the choriallantois into a placentome and a paraplacentome. Both the placentome and omphaloplacenta are organs of embryonic nutrition, but they transport nutrients by different mechanisms; the paraplacentome is a specialised gas exchange organ. The most placentotrophic species are in the South American genus *Mabuya*, where the females produce micro-lecithal eggs and the placenta is more complex than in any mammal, with four specialised structures for nutrient exchange and one for gas exchange. The number of independent origins of viviparity and the range of placental complexities exhibited by skinks enables us to infer the evolutionary trajectories that have resulted in microlecithal eggs and an almost complete reliance of placentotrophy from oviparous ancestors.

057

EUTHERIAN MAMMALS DO IT DIFFERENTLY: PLACENTAL ENDOCRINE STRATEGIES FOR THE MAINTENANCE OF PREGNANCY IN RODENTS AND PRIMATES

B. J. Waddell

School of Anatomy & Human Biology, The University of Western Australia, Nedlands, Australia

The placenta of rats and humans share important anatomical similarities, each with a chorio-allantoic, single discoid, haemochorial structure that facilitates highly efficient nutrient transport. Importantly, however, these similarities reflect convergent evolution and conceal markedly different developmental trajectories and endocrine functions. Placental endocrine signals are essential to drive maternal

adaptations that facilitate fetal development and ultimately successful birth. Central to these adaptations is a sustained increase in production of the sex steroids progesterone and oestrogen, each driven by very different placental signalling in rodents and primates. Specifically, while the rat placenta supplies androgen precursors for ovarian (luteal) oestrogen synthesis, in humans and closely-related primates the fetal adrenal cortex supplies androgen precursors for placental oestrogen synthesis. In both cases the resultant increase in oestrogen provides a local stimulus to ovarian (rat) and placental (primate) progesterone synthesis. This shift from a placental-ovarian to a feto-placental unit for oestrogen synthesis in primates may have evolved to ensure greater fetal influence over maternal adaptations. Placental regulation of maternal physiology is also mediated via a third steroid group, the glucocorticoids, which promote a successful pregnancy outcome via effects on maternal metabolism and fetal organ maturation. Glucocorticoids are produced within the HPA axis, activity of which is enhanced by the placenta (eg, via oestrogen in rodents and CRH in primates). Moreover, the placenta regulates access of maternal glucocorticoids to the fetus via expression of the 11 b -HSD enzymes which constitute the placental glucocorticoid barrier. Intriguingly, this barrier effectively disappears during late fetal life in rodents but increases markedly in primates (notably baboons and humans). We hypothesise that this opposite developmental change is due in part to the evolution of the feto-placental unit for oestrogen synthesis in these primate species, and the associated need to prevent suppression of the fetal HPA axis by maternal glucocorticoids in late gestation.

058

INSULIN RESISTANCE, TYPE 2 DIABETES, AND ASCVD: THE MISSING LINKS

R. De Fronzo

University of Texas Health Science Center, TX, United States

Insulin resistance is characteristic feature of type 2 diabetes mellitus (T2DM) and is associated with a metabolic and cardiovascular cluster of disorders (dyslipidaemia, hypertension, obesity glucose intolerance, hypercoagulability, inflammation, and endothelial dysfunction) known collectively as the insulin resistance (metabolic) syndrome. Each of these abnormalities is an independent risk factor for cardiovascular disease (CVD) and together they interact synergistically to increase the risk for CVD. However, collectively these CV risk factors can account for no more than 70% of the increased risk for ASCVD in subjects with T2DM. Multiple prospective studies (Botnia, IRAS, SAHS, Framingham study, Verona Diabetes study, Bruneck study) have documented an association between insulin resistance and accelerated CVD in patients with T2DM, as well as in non-diabetic individuals. At the molecular level, the basic etiology of the insulin resistance, i.e. impaired insulin signalling through the phosphoinositol-3 kinase pathway with intact signaling through the mitogen-activated protein kinase pathway, are responsible for the impairment in insulin-stimulated glucose metabolism but also contribute to the accelerated rate of CVD in T2DM. The same insulin signaling defect that is present in skeletal muscle also is present in coronary arterial smooth muscle cells. The current epidemic of diabetes is being driven by the epidemic of obesity, which represents a state of tissue fat overload. Accumulation of toxic lipid metabolites (fatty acyl CoA, diacylglycerol, ceramides) in muscle, liver, beta cells and arterial tissues contributes to the insulin resistance, beta cell dysfunction and accelerated atherosclerosis, respectively, in type 2 diabetes. Treatment with thiazolidinediones mobilizes fat out of tissues, leading to enhanced insulin sensitivity, improved beta cell function and decreased atherogenesis. Insulin resistance and lipotoxicity represent the missing links (beyond the classical cardiovascular risk factors) that help explain the accelerated rate of CVD in type 2 diabetic patients.

059

GESTATIONAL DIABETES AND TYPE 2 DIABETES IN PREGNANCY IN AUSTRALIA

N. Cheung

Centre for Diabetes & Endocrinology Research, Westmead Hospital, Westmead, NSW, Australia

In the last 30 years, there has been a dramatic increase in the incidence of gestational diabetes (GDM) in Australia. GDM has become a significant population health issue and Australia has been at the forefront of international research into its significance and management. More recently, the tsunami of GDM has been followed by a growing wave of type 2 diabetes in pregnancy. Type 2 diabetes is becoming more prevalent than type 1 diabetes in pregnancy, and adverse pregnancy outcomes are more common. However, diabetes itself is but one factor influencing outcomes in this group of women, with obesity, cultural issues and socioeconomic disadvantage being other significant issues.

The research of our group has focused on examining traditional and non-traditional risk factors for GDM, and for the progression from GDM to type 2 diabetes in Australia. Our research has also been directed towards breaking the nexus between GDM and type 2 diabetes. The identification of women with GDM is an opportunity to institute interventions to prevent both GDM and type 2 diabetes. Unfortunately there are numerous barriers to improving lifestyle and reducing diabetes risk in this population.

The National Diabetes Services Scheme has provided the opportunity to start translating some of our research into health promotion activities. The NDSS has greatly aided the management of diabetes and GDM by providing subsidised diabetes related products. It has also been established to provide information and services to people with diabetes. As part of this charter, the NDSS has recently started health promotion activities in the area of diabetes in pregnancy. It will underpin a national recall and screening program for diabetes after GDM, and forms the basis for other public health initiatives such as providing information to women with diabetes in pregnancy, facilitating the prevention of diabetes after GDM.

060

CALCIUM AND VITAMIN D ARE FIRST LINE EFFECTIVE OSTEOPOROTIC FRACTURE PREVENTION INTERVENTIONS

R. L. Prince

University of Western Australia, Nedlands, WA, Australia

The most common cause of osteoporotic fracture in Australia is gonadal hormone deficiency and aging in individuals with low peak bone mass due to genetic factors. This condition is associated with an increase in the dietary calcium requirement due to the loss of estrogen action to increase intestinal calcium absorption and reduce renal calcium excretion. In addition it is now clear that sunlight deficiency causing vitamin D deficiency is rampant in Australia due to the widespread adoption of an indoor lifestyle. Correcting vitamin D deficiency addresses the major problem of subclinical osteomalacia identified in Western populations again recently. It also corrects the long recognised proximal myopathy and thereby reduces the rate of falling by 25% in winter. Falling is a major component of the vast majority of appendicular fractures.

It is true that the quality of many of the calcium and vitamin D intervention studies is poor thus biasing the studies against finding an effect of calcium and vitamin D. The investigators running Record, one of the largest, did not meet most subjects face to face and were not able to ascertain compliance with the protocol regarding consumption of the tablets. The investigators state that even without tablet counting self reported consumption compliance was no higher than 50 %. Thus a negative result is to be expected. These problems with investigator initiated studies are often due to lack of proper funding for these studies and inexperienced investigators who do not know how to run such studies properly. Contrast this with a Pharmaceutical sponsored study where a huge amount of money and effort goes into retention and compliance by face to face meetings and tablet counting.

Next Pharmaceutical agents licensed for use in Australia combined with calcium and vitamin D have been shown to be more effective than calcium and vitamin D for fracture prevention only in individuals selected for low bone density or spinal fracture or both. The trial data are not generalisable to other groups such as those with minimal trauma fracture only without low bone density. Indeed a trial of risedronate in patients selected to be at increased risk of hip fracture due to other clinical risk factors conclusively demonstrated that risedronate was not effective in preventing hip fracture in this group.

In contrast the beneficial effect of calcium supplements especially with vitamin D have been demonstrated in whole of population interventions in which there has been demonstrated prevention of or reduction in the rate of bone loss. In addition in unselected whole population clinical trials total clinical minimal trauma fracture rates are reduced by 12% by the use of calcium with or without vitamin D, but not with vitamin D alone. Calcium and vitamin D have also been shown to reduce hip fracture risk by 18% in population based studies. While pharmaceutical agents have shown superiority this is in patients with osteoporosis or previous spinal fracture. Indeed in individuals with spinal fracture pharmaceutical agents should be first line because the data for spinal fracture does not in general show benefit from calcium and vitamin D.

In conclusion the trial data show that calcium and vitamin D are effective in reducing appendicular fracture and hip fracture rates in unselected elderly individuals. Pharmaceutical agents should be prescribed in the large number of individuals with age related and post menopausal osteoporosis where these agents have clearly been demonstrated to be superior to calcium and vitamin D alone.

061

CALCIUM AND VITAMIN D EFFICACY: WHAT ARE THE FACTS?

G. Jones

Menzies Research Institute, University of Tasmania, TAS, Australia

It is abundantly obvious that an adequate intake of calcium and vitamin D are required for optimal skeletal health. Despite this, there are many uncertainties about this area. Balance studies in children and adults indicate that virtually none are getting recommended calcium intakes and many are vitamin D insufficient. Yet attempts to increase calcium intake dramatically through supplements in children do very little to bone mass and calcium monotherapy in adults has not been proven to convincingly decrease fracture risk in very large meta-analyses. Recent balance studies incorporating varying calcium intakes have suggested that calcium balance can be achieved at much lower intakes calling into question the RDIs and indicating the time for a re-think. My view is that calcium should come from primarily dairy sources and low dose supplements should only be considered in dairy avoiders. Vitamin D is more confusing as there is less evidence and many methodological shortcomings with the original trials. We do not know what level to treat or to aim for. There are meta-analyses suggesting a dose above 800IU daily is ideal for falls and fractures but, in individual trials, this has only been proven to work in institutionalised setting where all are severely deficient. This suggests that increasing levels from 20 to 50nmol/l gives much more benefit that increasing levels from 40-80nmol/l. Indeed, a recent study has suggested achieving a level over 100nmol/l may actually be detrimental. Primum non nocere is a key principle in medicine. In the case of calcium supplements, the benefits do not appear to outweigh the downsides while this seems more applicable to vitamin D. In the case of those at higher fracture risk, neither is sufficient either alone or in combination although adequate vitamin D (but not calcium) seems necessary for bisphosphonates to work.

062

SAFETY OF CALCIUM AND VITAMIN D IN OSTEOPOROSIS

I. Reid

University of Auckland, Auckland, New Zealand

Calcium supplementation is widely used for the prevention of osteoporosis in postmenopausal women and in men, and has been regarded as safe. The recent finding of the Auckland Calcium Study that myocardial infarctions were more common in women randomised to calcium (BMJ 336:262), calls this assumption into question.

We have now reported a meta-analysis of placebo-controlled trials of calcium supplementation (>500 mg/day) with >100 participants and duration >1 year. 15 trials were eligible for inclusion, with patient-level data available for 5 studies (N=8151), and trial-level data for 11 studies (N=11921). HRs for myocardial infarction in those allocated to calcium were 1.31(1.02-1.67) and 1.27 (1.01-1.59), respectively, in these 2 groups of trials. Mean follow-up was 4 years.

Cardiovascular (CVS) event data from the WHI have been published, and show an interaction between BMI and the CaD intervention. In non-obese women, the HR of MI or coronary revascularization was 1.24 (1.09-1.42) in those allocated to CaD. This study is also complicated by use of non-study Ca supplements in 56% of subjects. In those not self-administering Ca, the HR of this composite endpoint was 1.17 (1.01-1.36). Calcium supplements also increase vascular risk in renal patients.

Epidemiological evidence shows that high-normal serum calcium levels are a risk factor for vascular disease, and calcium supplements acutely elevate serum calcium. Together, these might explainthe adverse effect of supplements on CVS risk. There should be a reappraisal of the role of calcium supplements in osteoporosis management.

063

SAFETY OF CALCIUM SUPPLEMENTS IN POSTMENOPAUSAL WOMEN

B. E.C. Nordin[1,2]

[1]Endocrine and Metabolic Unit, Royal Adelaide Hospital, Adelaide, SA, Australia
[2]Medicine, University of Adelaide, Adelaide, SA, Australia

Calcium supplementation has been used in the prevention and treatment of osteoporosis for some 50 years without any serious concern about their safety until very recently when a New Zealand group suggested from data of borderline significance that it might increase the risk of cardiovascular disease and recommended the use of bisphosphonates instead [1,2]. They do not consider that there is a risk from dairy-product calcium or calcium with vitamin D but only from calcium mono-therapy. This concept will be reviewed in the light of evidence from current literature, but since most calcium supplements are now given with vitamin D, it may not be possible to confirm or refute the hypothesis beyond all doubt.

(1) Bolland MJ, et al. Vascular events of healthy older women receiving calcium supplementation: randomised controlled trial. BMJ 2008;3436:262-269
(2) Reid IR, Bolland MJ, Grey A. Does calcium supplementation increase cardiovascular risk? Clinical Endocrinology (accepted for publication January 2010)

201

LOW FREE TESTOSTERONE PREDICTS FRAILTY IN OLDER MEN

Z. Hyde[1,2], L. Flicker[1,2], O. P. Almeida[1,3,4], G. J. Hankey[2,5], K. A. McCaul[1,2], S. A. Paul Chubb[2,6], B. B. Yeap[2,7]

[1]Western Australian Centre for Health and Ageing, Centre for Medical Research, Western Australian Institute for Medical Research, Perth, WA, Australia
[2]School of Medicine and Pharmacology, University of Western Australia, Crawley, WA, Australia
[3]School of Psychiatry and Clinical Neurosciences, University of Western Australia, Crawley, WA, Australia
[4]Department of Psychiatry, Royal Perth Hospital, Perth, WA, Australia
[5]Stroke Unit, Royal Perth Hospital, Perth, WA, Australia
[6]PathWest, Department of Biochemistry, Fremantle Hospital, Fremantle, WA, Australia
[7]Department of Endocrinology and Diabetes, Fremantle Hospital, Fremantle, WA, Australia

Background: The prevalence of frailty increases, whilst testosterone decreases as men age. Low testosterone may be a risk factor for the development of this syndrome.

Aim: We aimed to determine whether testosterone levels are associated with frailty.

Methods: Between 2001-04, frailty was assessed in 3,616 community-dwelling men aged 70-88 years. Frailty was re-assessed in 1,586 men aged 76-93 years in 2008-09. Frailty was assessed with the FRAIL scale, comprising 5 domains: fatigue; difficulty climbing a flight of stairs; difficulty walking more than 100 metres; more than 5 illnesses present; or weight loss greater than 5%. Testosterone, sex hormone binding globulin (SHBG) and luteinizing hormone (LH) were assayed at baseline. Free testosterone was calculated using mass action equations.

Results: At baseline 15.2% of men (n=548) were frail (≥3 deficits), increasing to 23.0% (n=364) at follow-up. At baseline, each 1 standard deviation decrease in total or free testosterone levels was associated with increased odds of frailty (odds ratio [OR]=1.23; 95% confidence interval [CI] 1.11, 1.38 and OR=1.29; 95% CI 1.15, 1.44 for total and free testosterone respectively). Lower LH was associated with reduced odds of frailty (OR=0.88; 95% CI 0.81, 0.95). Adjustments were made for age, body mass index, smoking, diabetes, social support and other covariates. At follow-up, only lower free testosterone levels (OR=1.22; 95% CI 1.05, 1.42) predicted frailty.

Summary and Conclusions: Lower free testosterone was independently associated with frailty cross-sectionally and longitudinally. Randomised trials should explore whether testosterone therapy can prevent the development of frailty.

202

PROGESTERONE AND ELF5 IN MAMMARY GLAND DEVELOPMENT

H. J. Lee[1], C. J. Evans[1], A. Boulghourjian[1], J. P. Lydon[2], C. J. Ormandy[1]

[1]Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia
[2]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas, Australia

During pregnancy the mammary gland undergoes hormonally regulated changes as lobuloalveoli form along the ductal network. Elf5 is a key transcription factor that specifies alveolar cell fate in the mammary gland during pregnancy (1). The ability of Elf5 to rescue the failed development of prolactin (PRL) receptor null mammary glands has established a major role for Elf5 in mediating PRL action during pregnancy (2). We have recently reported that Elf5 is also regulated by progesterone (P) in cell lines and mice (3), raising the possibility that Elf5 co-ordinates both P and PRL actions. The aims of this study were to determine whether Elf5 mediates P action, and to investigate how P and Elf5 co-operate during mammary development.

Elf5 transgenic mice were crossed with PRLacZKI animals to allow induction of Elf5 in mammary glands null for PR, and transplantation studies were used to assess the development of these glands during pregnancy. To study the combined effects of Elf5 and P, Elf5 transgenic mice were implanted with a sub-cutaneous slow-release P pellet and Elf5 expression was induced by doxycycline administration.

Induction of Elf5 did not rescue the failed development of PR null transplants, indicating that Elf5 is not primarily responsible for P action during pregnancy. However, Elf5 and P had additive effects on endpoints of mammary gland differentiation. Most notably, in the presence of P, Elf5 promoted the formation of clusters of polarised alveolar buds. This phenotype resembles that achieved by over-expression of RANKL (4), a mediator of P's paracrine actions. We also report that PR and Elf5 are expressed in neighbouring mammary cells, consistent with the idea that P induces Elf5 expression via a paracrine mechanism. In conclusion, Elf5 co-operates with P to promote alveolar bud formation. Further work will investigate whether RANKL mediates P induction of Elf5 expression.

(1) Oakes et al (2008) The Ets transcription factor Elf5 specifies mammary alveolar cell fate. Genes Dev 22(5): 581-6.
(2) Harris et al (2006) Socs2 and Elf5 mediate prolactin-induced mammary gland development. Mol Endocrinol 20(5): 1177-1187.
(3) Hilton et al (2010) The anti-proliferative effects of progestins in T47D breast cancer cells are tempered by progestin-induction of the ets transcription factor Elf5. Mol Endocrinol (in press).
(4) Fernandez-Valdivia et al (2009) The RANKL signaling axis is sufficient to elicit ductal side-branching and alveologenesis in the mammary gland of the virgin mouse. Dev Biol 328(1): 127-39.

203

ESTROGEN RECEPTOR B ACTIVATES TNFA-MEDIATED APOPTOSIS IN BENIGN PROSTATIC HYPERPLASIA

S. Hussain[1], S. J. Mcpherson[1], P. Balanathan[1], S. L. Hedwards[1], M. Grant[1], Y. Wang[2,3], R. A. Taylor[1], G. P. Risbridger[1]

[1]Prostate & Breast Cancer Research Program, Department of Anatomy and Development, Monash University, Clayton, VIC, Australia
[2]Department of Urologic Sciences, The Vancouver Prostate Centre, UBC, Vancouver, British Columbia, Canada
[3]Department of Cancer Endocrinology, BC Cancer Agency, Vancouver, British Columbia, Canada

Benign prostatic hyperplasia (BPH) is the most common benign neoplasm in men, characterised by nodule formation in stroma and epithelia of the prostate gland. Prostatic hyperplasia leading to bladder dysfunction or obstruction occurs in ~80% of men over 70 years old. Current treatments include androgen blockade, pharmaceutical or surgical management. However selective targeting of stromal and epithelial cell populations implicated in the etiology of BPH (including androgen independent epithelial cells), remains a significant challenge.

Here we show a selective estrogen receptor β (ERβ) agonist targets both stroma and androgen independent basal epithelial cells (including putative stem/progenitor cells), causing apoptosis that is mechanistically different to castration; including activation of the extrinsic apoptotic pathway via TNFα mediated signalling. The significant decline in basal cell number after exposure to ERβ agonist perturbs secretory function and ductal structure during recovery. ERβ agonist also causes epithelial and stromal apoptosis in human xenografted BPH specimens, as well as in benign (BPH-1, RWPE) cell lines, including a subpopulation of α2β1-integrinhi/CD133+ cells with proven regenerative potential.

Our studies show that ERβ activates apoptosis in BPH via TNFα-mediated signaling. This is the first evidence of an androgen-independent pro-apoptotic action of ERβ agonist that is mechanistically different to castration. Prostatic stroma and epithelia including castrate-resistant p63+ and CD133+ basal cells are cellular targets of ERβ activation. These pre-clinical studies support the rationale for the potential clinical application of ERβ agonists, either alone, or in combination with existing androgen blockade, for the treatment of BPH.

204

THE ROLE OF RABL2A IN PROTEIN TRAFFICKING AND ITS RELATION WITH MALE FERTILITY AND CILIOPATHIES

C. Lo[1], D. Jamsai[1], C. Borg[1], C. Ormandy[2], M. O'Bryan[1]

[1]Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia
[2]Garvan Institute of Medical Research, Sydney, NSW, Australia

Cilia are hair-like structures found on the surface of a wide range of cell types. In motile cilia (flagella), such as the sperm tail, the nine peripheral microtubules of the axoneme have two additional central microtubules (9+2), while primary cilia lack the central tubules (9+0). Genetic dysfunction of motile cilia results in the syndrome Primary Ciliary Dyskinesia, which is characterized by a range of symptoms including male infertility and respiratory congestion. In more than 60% of the Primary Ciliary Dyskinesia cases, the aetiology remains unknown.

ENU mutagenesis in mice produced a mouse line showing a phenotype that models Primary Ciliary Dyskinesia. Males have qualitatively normal spermatogensis, but reduced daily sperm output and asthenospermia resulting in sterility. Lung pathology indicates congestion. Our lab has successfully identified a point mutation in the *Rabl2a* gene in this mouse line. The causal mutation was defined as an A to G substitution in the first base of exon 5 of *Rabl2a,* which resulted in substitution of an aspartic acid for a glycine in two of the three RABL2A isoforms. In gene expression analysis, *Rabl2a* is expressed in various ciliated tissues including lungs, trachea, oviduct and testis. RABL2A is an uncharacterized member of the RAS superfamily, a family of GTPases. Many Ras proteins are involved in membrane trafficking and cell signalling. Active GTP-bound RAB protein recruits effector proteins to be delivered to membranes. RABL2A protein is immuno-localized on the sperm flagellum principal piece suggesting that RABL2A plays a role in delivering proteins to cilia membranes. Collectively these data suggest that RABL2A is critically involved in sperm formation and motility, and potentially in the formation of other cilia types. Mutation or dysregulation of RABL2A may be implicated in ciliopathies and provide the basis for the 60% of the Primary Ciliary Dyskinesia cases for which the aetiology remains unknown.

205

IMMUNISATION WITH OXIDISED LDL PROTECTS AGAINST INSULIN RESISTANCE IN DIETARY-INDUCED OBESITY

N. Kasmeridis, D. Girard, N. Petrovsky

Department of Endocrinology, Flinders Medical Centre, Bedford Park, SA, Australia

Background: Immunisation with oxidised low density lipoprotein (oxLDL) has previously been shown to have anti-atherosclerotic effects although the mechanism for this protection remains unclear. Insulin resistance may play a major causal role in atherosclerosis. In this study we sought to test whether oxLDL immunisation might reduce obesity-associated insulin resistance.

Method: LDL was isolated from mice serum and oxidised with copper sulfate. C57BL/6J mice were fed a high fat diabetogenic diet and were immunised at week 3, 5, 7, 11 and 15 with oxLDL (50μg) (n=7) or saline control group (n=8). At wk 26, insulin tolerance (ITT) and intraperitoneal glucose tolerance tests (GTT) were performed and serum collected for fasting glucose and insulin. Results: Immunisation with oxLDL reduced fasting glucose and insulin levels and measures of insulin resistance (Homeostasis Model Assessment of Insulin Resistance) in obese C57BL/6J mice. Mice immunised with oxLDL had a significantly lower HOMA score (mean 3.6; SD 0.9) than control mice (6.9:3.1), $p<0.05$. The reduction in insulin resistance in the oxLDL immunised mice was not explainable by weight differences, as there was no significant differences in weight between oxLDL (36.5g;1.7) and control (37.5g;3.7) mice on the high fat diet.

Conclusion: Immunisation with oxLDL protected against obesity-induced insulin resistance. Given that insulin resistance is a major risk factor for atherosclerosis, these findings may explain how oxLDL immunisation protects against atherosclerosis. Further investigation is warranted of oxLDL immunisation strategies against type 2 diabetes and atherosclerosis.

206

ATYPICAL FEMUR FRACTURES AND BISPHOSPHONATE USE

C. M. Girgis[1], D. Sher[2], M. J. Seibel[1]

[1]Endocrinology and Metabolism, Concord Repatriation General Hospital, Sydney, NSW, Australia
[2]Orthopaedic Surgery, Concord Repatriation General Hospital, Sydney, NSW, Australia

Background: Reports have suggested an association between the use of bisphosphonates and the occurrence of atypical femur fractures. These fractures are morphologically distinct from the more common, osteoporotic hip fracture and are characterised by particular radiographic features and their subtrochanteric location. (1) Aim: To determine the incidence of atypical fractures and their relationship with bisphosphonate use. Methods: We reviewed the X-rays of 152 non-hip femoral fractures sustained by 152 patients (mean age 78±5 years) admitted to a tertiary centre between June 2003 and May 2008. A senior orthopedic surgeon, blinded to the patients' characteristics and medication, reviewed the fracture radiographs of every patient in random sequence on two separate occasions (Cohen's $\kappa = 0.8$), identifying those fractures that displayed the criteria for an atypical fracture as previously described. (1) Results: Twenty of the 152 fractures were identified as atypical. Seventeen of these 20 patients were on oral bisphosphonates (15 alendronate, 2 risedronate, mean duration 5.1 and 3yrs, respectively). Of the 132 patients whose fractures did not fulfill the criteria of an atypical fracture, 2 were on alendronate (1.5%) and 1 patient was on risedronate (0.76%; mean treatment duration 3.5yrs and 1yr, respectively). The atypical fracture pattern was 96.7% specific to bisphosphonate users. Additional risk factors included previous low-energy fractures, glucocorticoid therapy, active rheumatoid arthritis and low serum 25-hydroxy-vitamin D levels. The estimated annual incidence of atypical fractures was 0.23/10,000 in the general population served by our institution and 1.6/10,000 in people above 65 years of age. Using data on the wholesale purchase of alendronate and risedronate by pharmacies in the hospital's catchment area, the estimated annual incidence of atypical fractures was 10/10,000 in alendronate users and 3/10,000 in risedronate users. Conclusion: While there seems to be an association between atypical femur fractures and oral bisphosphonate use, these fractures are rare and the anti-fracture effects of bisphosphonates outweigh the potential risk.

(1) Lenart et al. N Engl J Med 2008;358:1304-6

207

INVESTIGATING NON-GENOMIC SIGNALLING PATHWAYS OF THE ANDROGEN RECEPTOR

T. P.S. Pang, R. A. Davey, M. V. Clarke, H. E. MacLean

Medicine (AH/NH), University of Melbourne, Heidelberg, VIC, Australia

Activated androgen receptor (AR) binds DNA and regulates gene expression, but may also have DNA binding-independent actions, including indirect gene repression [1] and activation of ERK/CREB signalling [2]. We are using our AR knockout (ARKO) mouse model, which has deletion of AR DNA binding activity, to investigate DNA binding-independent AR actions.

We previously showed that AR expression is normal in most ARKO tissues, but up-regulated in muscle. We now show that AR protein and mRNA levels are normal in cultured genital skin fibroblasts (GSFs) from ARKO males. Using ARKO GSFs, in which we have previously showed that mutant AR binds ligand normally, we now demonstrate the mutant AR has reduced nuclear translocation following androgen-binding by immunocytochemistry. Both wildtype and ARKO GSFs have increased ERK phosphorylation following 1 min 100 nM DHT treatment ($p<0.05$) and this is abolished by the AR antagonist bicalutamide ($p<0.001$). Preliminary data show that the genomic AR target gene, *ApoD*, is regulated by DHT in wildtype but not ARKO GSFs, and has lower expression in ARKO cells. The non-genomic AR target gene, *Ngfr*, is down-regulated by DHT in wildtype GSFs and we are currently investigating its expression in ARKO cells. We are also currently examining the effect of orchidectomy ± DHT replacement in wildtype and ARKO male mice. Preliminary data show that the expression of the genomic AR target gene *Odc1*, is up-regulated by androgens in wildtype but not ARKO kidney. In addition, we are currently investigating non-genomic target gene expression and phosphorylation of second messenger pathways in these mice.

This study has characterised the function of mutant AR expressed in ARKO mice and examined the expression of several androgen-responsive genes. Further characterisation of androgen effects in orchidectomised ARKO males will determine the physiological relevance of non-DNA binding-dependent actions of the AR.

(1) Kallio PJ et al. J. Mol. Endo. 9: 1017-1028, 1995
(2) Unni E et al. Cancer Research 64:7156-7168, 2004

208

THE GLUCOCORTICOID RECEPTOR IS OVEREXPRESSED AND TRANSCRIPTIONALLY ACTIVE IN MALIGNANT ADRENOCORTICAL TUMOURS

L. Tacon[1,2], P. Soon[1], N. Armstrong[3], B. Robinson[1,2], S. Sidhu[1], R. Clifton-Bligh[1,2]

[1]Cancer Genetics Unit, Hormones & Cancer Group, Kolling Institute of Medical Research, Sydney, NSW, Australia
[2]Department of Endocrinology, Royal North Shore Hospital, Sydney, NSW, Australia
[3]Garvan Institute of Medical Research, Sydney, NSW, Australia

Objectives: Adrenocortical carcinoma (ACC) is an aggressive malignancy with a poor prognosis. We have used microarray profiling to elucidate molecular markers that may offer diagnostic, prognostic or therapeutic utility. We recently demonstrated that *NR3C1*, the gene encoding the glucocorticoid receptor (GR), was overexpressed in ACCs, $p<0.001$, and that immunohistochemistry for GR demonstrated positive nuclear staining in 31/33 ACCs (95%) and negative staining in 40/41 adrenocortical adenomas (98%), $p<0.001$[1]. The objective of this study was to study GR expression in adrenocortical tumours (ACTs) and to use a cell line model to investigate transcriptional targets of GR in adrenocortical cancer cells.

Methods: Microarray analysis, qPCR, Western blot, direct sequencing and immunohistochemistry were performed. The human adrenocortical carcinoma cell line, H295R, was utilised to induce stable overexpression of GRα. Microarray profiling was performed on RNA isolated from H295R_GRα cells at baseline and after 6h dexamethasone 10^{-7}M treatment.

Results: Microarray profiling of H295R_GRα cells demonstrated a significant cohort of genes to be differentially expressed at baseline and after dexamethasone treatment. Comparison of these *in vitro* profiling results with our ACT microarray results identified individual targets and KEGG pathways that were common to both experiments. The differential expression of key individual transcripts was validated by qPCR in both H295R_GRα cells and ACCs, confirming the overexpression of *CCNB2*, *CASC5* and *BUB1B* mRNA, and underexpression of *NOV, NPY1R* and *HSD3B2* mRNA. KEGG pathway analysis identified ECM-receptor interaction and focal adhesion pathways as overrepresented in H295R_GRα cells and in ACCs, while pathways involved in metabolic processes were underrepresented in both H295R_GRα cells and ACCs.

Conclusions: Nuclear GR staining by immunohistochemistry may complement existing algorithms in ACC diagnosis. GR demonstrates complex transcriptional activity in adrenocortical cells, including modulation of genes involved in neoplasia. We suggest that GR expression may be central to ACC pathogenesis and represent a new therapeutic target.

(1) Tacon LJ, et al., 2009 J Clin Endocrinol Metab 94:4591-4599

209

'CAPTURING' FRACTURED PATIENTS: IMPROVING MANAGEMENT OF OSTEOPOROSIS IN A TERTIARY HOSPITAL

T. D.T. Vu, S. Iuliano-Burns, C. Chiang, E. Gianatti, E. Hamilton, M. Grossmann, E. Seeman, J. Zajac

Medicine, University of Melbourne, Heidelberg, VIC, Australia

Background: A recent audit at Austin Health indicated that <1% and 6% of patients admitted with fragility fractures received appropriate medical therapy and follow-up respectively.

Aims: The "Fracture Capture Project" was initiated to develop and implement evidence based guidelines in the management of fragility fractures.

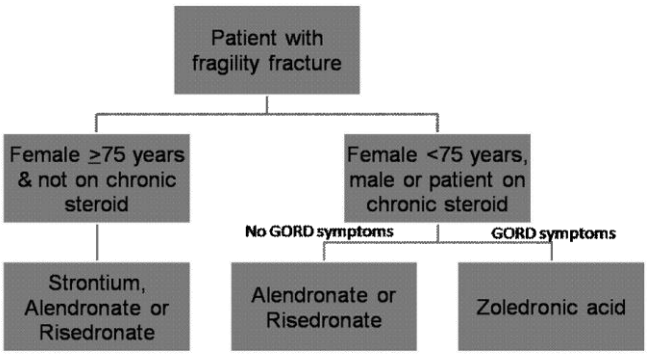
Methods: Patients with fragility fractures were identified weekly through the Emergency Department. Inpatients had clinical assessment and biochemical investigations for metabolic bone disease. Treatment (calcium, vitamin D and bisphosphonate or strontium) was commenced according to guidelines (figure 1.). Endocrine clinic review was scheduled following an outpatient DXA. Patients that were discharged from the Emergency Department or before inpatient assessment were mailed secondary osteoporosis screen and DXA referral forms and an endocrine clinic appointment. Reminder letters were sent to patients who did not initially respond.

Results: Over 12 months 481 females, aged 74.8+11.4 years and 169 males, aged 71.1+11.8 years with fragility fractures were identified. Severe vitamin D deficiency (<25nmol/L) was detected in 9% while 29% were moderately (49-25nmol/L) and 40% were mildly (74-50nmol/L) deficient. DXA carried out in 25% of patients revealed that 47% had osteopenia and 26% had osteoporosis in the femoral neck.

Treatment (bisphosphonates or strontium) was commenced in 52% of inpatients compared to 0.8% reported prior to the intervention strategy. Calcium and vitamin D therapies were prescribed in 55% of inpatients compared to 12% reported in the audit. Factors associated with failed uptake of therapy included: discharge prior to inpatient assessment (32%), severe renal impairment (7%) and death (4%). Forty-three percent were booked into or have attended an endocrine clinic compared to 6% observed previously in the audit.

Conclusions: Implementation of a dedicated osteoporosis service improves initiation of therapy. Whether this translates into improved compliance and fracture risk reduction needs to be studied.

Figure 1. Treatment guidelines



GORD (gastro-oesophageal reflux disease)

210

OSTEOPOROSIS MEDICATION AND MORTALITY RISK IN ELDERLY WOMEN AND MEN: AN 18-YEAR PROSPECTIVE STUDY FROM DUBBO OSTEOPOROSIS EPIDEMIOLOGY STUDY

J. R. Center[1,2,3], D. Bliuc[1], N. D. Nguyen[1], T. V. Nguyen[1,3], J. A. Eisman[1,2,3]

[1]Osteoporosis & Bone Biology, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia
[2]St Vincent's Hospital, Sydney, NSW, Australia
[3]University of New South Wales, Sydney, NSW, Australia

Osteoporotic fractures are associated with premature mortality and with increased risk of subsequent fracture, which again raises mortality risk. While it is clear that osteoporosis treatment reduces the incidence of fracture, some studies suggest that it reduces mortality risk. The aims of this study were to examine the effect of osteoporosis treatment on 1) all cause mortality in women and men over 60+ and 2) post-fracture mortality in women.

There were 429 and 153 low trauma fractures (women and men, respectively) and 466 deaths over 15453 person-years in women and 400 deaths over 9522 in men amongst 1223 women and 819 men participating in the Dubbo Osteoporosis Epidemiology Study (April 1989-May 2007). Bone mineral density and information on co-morbidities and medications were obtained. Osteoporosis medication was classified as: bisphosphonates (BP), current hormone replacement therapy (HT) and calcium ± vitamin D only (CaD). Propensity matching was used to adjust for baseline differences.

There were 325 women (BP, n=106; HRT, n=77, CaD, n=142) and 37 men (BP, n=15; CaD, n=22) who received osteoporosis medication. The majority of those on BP had had a fracture (women, n=71; men, n=8).

In multivariate analyses, mortality risk was reduced for BP [HR: 0.3 (0.2, 0.6)] but not for HT [HR: 0.8 (0.4, 1.8)] and CaD [HR: 0.99 (0.76, 1.31)] in women compared with non-treated individuals. When limiting the analysis to the women with fracture, mortality risk was still reduced in the group treated with BP [adjusted HR: 0.33 (0.16, 0.66)]. In men, mortality risk was reduced to a similar extent for those on BP but was non-significant after adjustment for covariates [HR 0.48 (0.11, 1.98)].

These data suggest that bisphosphonates reduce the risk of all cause and post-fracture mortality in women and perhaps in men. These findings warrant further exploration in larger studies.

211

THE EFFECTS OF A TWO YEAR RCT OF WHEY PROTEIN SUPPLEMENTATION ON BONE DENSITY AND URINARY CALCIUM EXCRETION IN OLDER POSTMENOPAUSAL WOMEN

K. Zhu[1,2], X. Meng[3], D. A. Kerr[3], A. Devine[4], V. Solah[3], C. W. Binns[3], R. L. Prince[1,2]

[1]Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Perth, WA, Australia
[2]School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia
[3]School of Public Health, Curtin University of Technology, Perth, WA, Australia
[4]School of Exercise, Biomedical and Health Sciences, Edith Cowan University, Perth, WA, Australia

Aims of study: Epidemiological studies including our own (1) and randomized trials in frail women after hip fracture have suggested that increased protein intake may reduce bone loss in elderly women. To evaluate this, a 2-year protein supplementation RCT was performed.

Methods: 219 healthy ambulant women aged 70-80 years were randomized to either a high protein (n=109) or low protein drink (n=110) both of which consisted of a 250 ml drink providing 600 mg of calcium and 3.3 kJ/ml of energy. The high protein drink had 30 g of whey protein and the low protein drink had 1.7 g protein. DXA total hip BMD and 24h urinary calcium excretion was measured at baseline and year 2.

Results: Baseline protein intake was 76.2 ± 18.0 g/day (1.14 ± 0.33 g/kg body weight/day). There was a significant decrease in total hip BMD at year 2 in both the protein (-11 ± 3 mg/ cm^2) and placebo (-8 ± 2 mg/ cm^2) groups but there was no between group difference. At baseline 24h urine calcium did not differ in the two groups but there was a significant increase over the two years in the protein group (0.53 ± 1.62 mmol/day) compared to the placebo group (-0.07 ± 1.57 mmol/day, P = 0.14).

Conclusion: Although the high protein drink produced the expected increase in urine calcium there was no detrimental or beneficial effect on hip bone mass. In these healthy ambulant women with baseline protein intake above current Estimated Average Requirement of 0.75g/kg body weight/day, extra protein was not a critical beneficial or deleterious regulator of their bone mass.

(1) Meng X, et al. J Bone Miner Res 2009;24:1827-1834.

212

DENOSUMAB (DMAB) EFFECTS ON BONE MINERAL DENSITY (BMD) AND FRACTURE STRATIFIED BY BASELINE LEVEL OF RENAL FUNCTION

P. R. Ebeling[1], S. A. Jamal[2], O. Ljunggren[3], C. Stehman-Breen[4], S. R. Cummings[5], M. R. McClung[6], S. Goemaere[7], E. Franek[8], Y. Yang[4], O. I. Egbuna[4], P. Miller[9]

[1]The University of Melbourne, Footscray, Australia
[2]University of Toronto, Toronto, Canada
[3]Uppsala University Hospital, Uppsala, Sweden
[4]Amgen Inc., Thousand Oaks, CA, United States
[5]University of California, San Francisco, CA, United States
[6]Oregon Osteoporosis Centre, Portland, OR, United States
[7]Ghent University Hospital, Ghent, Belgium
[8]Central Clinical Hospital, Poland
[9]University of Colorado Health Sciences Center, CO, United States

Introduction Osteoporosis and chronic kidney disease (CKD) both increase with ageing, yet few data exist on anti-osteoporosis treatments in CKD. We examined the efficacy and safety of DMAb among 7868 postmenopausal women with osteoporosis and varying levels of renal function participating in the FREEDOM Study; a 3 year, randomized placebo controlled trial.

Methods We estimated creatinine clearance (eGFR) using the Cockcroft-Gault equation and classified baseline levels of renal function using the modified National Kidney Foundation CKD classification. We examined incident fracture rates, changes in BMD, serum calcium, creatinine and adverse events in subjects receiving DMAb and placebo, stratified by level of renal function, using linear regression models adjusted for fracture since aged 45, prevalent vertebral fractures, self-reported health status, baseline calcium intake, current smoking, femoral neck BMD T score, and years since menopause. We used a subgroup interaction term to determine if there were differences in treatment effect by eGFR.

Results Mean age was 72.3 ±5.2 years, mean weight was 63.8 ± 10.4 kg, serum creatinine was 70.8 ± 15.3mmol/L and calcium was 2.44 ±0.11mmol/L. 73 women had an eGFR between 15 to 30ml/min; 716 between 30 to <45ml/min; 2101 between 45 to <60ml/min and 4911 had an eGFR of ≥ 60ml/min.Vertebral and nonvertebral fracture risk reduction, as well as the difference in the mean % changes in BMD in subjects treated with DMAb compared with placebo, did not differ by level of renal function, and were in favour of DMAb. The test for treatment by subgroup interaction was not statistically significant indicating fracture risk reduction at all levels of eGFR. Changes in creatinine, calcium, and the incidence of adverse, serious adverse and fatal events were similar between groups and did not differ by level of renal function.

Conclusion DMAb reduces fracture risk without increasing adverse events in patients with CKD.

213

INCREASED FRACTURE RISK IN PATIENTS WITH NEWLY DIAGNOSED TYPE 1 DIABETES MELLITUS MAYBE PARTLY DUE TO REDUCED CORTICAL DENSITY

T. Vu[1,2], A. Ghasem-Zadeh[2], E. Seeman[1,2], G. Jerums[1,2]

[1]Endocrinology, Austin Health, Heidelberg West, VIC, Australia
[2]Medicine, University of Melbourne, Heidelberg, VIC, Australia

Patients with type 1 diabetes mellitus (T1DM) have a greater risk of fragility fracture than non-diabetics. However, bone density, determined using dual x ray absorptiometry, is often not reduced or only modestly reduced relative to controls. We hypothesized that abnormalities in bone micro-architecture may be responsible for the underlying bone fragility.

To address this hypothesis, we quantified bone micro-architecture using high-resolution peripheral computed tomography of the distal radius and tibia (Scanco) in 24 patients with type 1 diabetes (mean age 28, range 18-54 yrs) and 50 age and sex matched controls. Morphology was expressed as Z scores (mean + SEM).

As shown in the table, no deficits were detected in total volumetric bone mineral density (vBMD) at their site. However, cortical vBMD and trabecular thickness (but not number) were reduced, at both tibia and radius.

We infer that the increased risk for fracture, particularly ankle and hip fracture, may be the result of reduced cortical vBMD, which is likely to be due to increased intracortical porosity as well is reduced trabecular thickness, both may be due to the documented reduction in bone formation in patients with diabetes.

	Tibia		Radius	
	Diabetics	Controls	Diabetics	Controls
Total vBMD	326.400 + 11.32 0.12 + 0.23	312.89 + 5.99	339.37 + 13.76 0.15 + 0.24	329.81 + 6.67
Cortical				
Area	129.58 + 6.53 -0.36 + 0.24	133.75 + 3.43	62.53 + 2.90 -0.09 + 0.23	62.18 + 1.64
vBMD	878.13 + 7.15 -0.32 + 0.75	889.40 + 4.86	855.57 + 11.37 -0.28 + 0.20	887.44 + 5.20
Porosity				
Trabecular				
vBMD	210.08 + 9.08 0.33 + 0.20	185.53 + 5.13	191.83 + 9.74 0.31 + 0.22	166.31 + 4.74
Number	1.88 + 0.08 -0.32 + 0.24	2.07 + 0.04	1.86 + 0.06 -0.28 0.82	1.95 + 0.04
Thickness	0.09 + 0.00 -0.66 + 0.31	0.07 + 0.00	0.09 + 0.00 0.11 +0.26	0.07 + 0.00

214

CALCIUM SUPPLEMENTATION AND THE RISK OF ATHEROSCLEROTIC VASCULAR DISEASE IN POSTMENOPAUSAL WOMEN

J. R. Lewis[1,2], J. Calver[3], K. Zhu[1,2], L. Flicker[2,3], R. L. Prince[1,2]

[1]Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia
[2]School of Medicine and Pharmacology, University of Western Australia, Nedlands, WA, Australia
[3]Centre for Health and Ageing, University of Western Australia, Nedlands, WA, Australia

Aims of the study: Concern has been expressed that calcium supplementation, a key public health intervention for preventing osteoporotic fracture in older women, may increase the risk of atherosclerotic vascular disease (ASVD). The risk was evaluated by examination of verified data on ASVD hospitalisation and mortality from a 5-year RCT of calcium carbonate and 4·5-year post-trial follow-up.

Methods: Complete hospital admission and mortality data were obtained from the Western Australian Data Linkage Service (WADLS), which provides 100% ascertainment and ICD coding of all events in Western Australia, for patients recruited to the 5-year, randomised double-blinded placebo controlled trial (Calcium Intake Fracture Outcome Study, CAIFOS). 1,460 female participants aged 75·1 ± 2·7 years were recruited and randomised to receive 1,200 mg of calcium carbonate daily or an identical placebo.

Results: In the 5-year *intention-to-treat* analysis 104 participants (31·4/1000 person years) in the calcium supplementation group and 103 (30·9/1000 person/years) in the placebo group sustained either hospitalisation or death from ASVD; age-adjusted ITT HR 1·005 95% CI 0·766-1·320, all covariate-adjusted ITT HR 0·938 95% CI 0·690-1·275. At 9·5 years, 195 participants (33·9/1000 person years) in the calcium supplementation group and 200 participants (34·5/1000 person years) in the placebo group sustained hospitalisation or death from ASVD; age adjusted ITT HR 0·975 95% CI 0·800-1·187, all covariate adjusted ITT HR 0·919 95% CI 0·737-1·146.

Conclusion : This trial provides compelling evidence that calcium supplementation of 1,200 mg daily does not significantly increase the risk of atherosclerotic vascular disease in elderly women.

215

IS THE PARITY INDUCED DECREASED BREAST CANCER RISK DUE TO A DECREASE IN ESTROGEN SENSITIVITY IN THE BREAST?

K. L. Britt, Y. Razavi, S. Hawthorne, G. P. Risbridger

Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia

Early parity (childbirth) decreases a woman's risk of developing estrogen receptor (ER) positive breast cancer (BC) by as much as 70% if she has her children before the age of 20 years. It is not clear how the breasts of these women are altered to induce such protection against BC. As pregnancy exposes the breast to estrogen levels 10-100 times higher than those experiences during normal cycling we hypothesized that the parity-induced protection against BC may be due to reduced estrogen sensitivity of the breast following exposure to high estrogen levels. To define this we assessed ER-alpha expression levels in the mammary glands of parous mice (that also exhibit parity-induced protection against BC) compared to age matched nulliparous (have borne no offspring) mice. In estrus stage matched samples of mouse

mammary glands we found that there was a decrease in ER-alpha protein expression (immunohistochemistry and immunofluorescence) throughout the parous gland. When stereologically assessed in isolated regions of the mammary gland (proximal, central and distal in location with respect to the nipples) the percentage of ER-alpha positive cells was significantly reduced in the distal tips of the parous mammary glands (17.5% in nulliparous and 9.1% in parous, p=0.03). Thus parity appears to have reduced estrogen sensitivity in mouse mammary glands. To determine if the decreased ER expression translates to a functional decrease in estrogen sensitivity we assessed the ability of estrogen to induce a proliferative response in the mammary gland. We modulated the assay of estrogen sensitivity (estrogen induced proliferation) which was used to define the estrogen responsiveness in pre-pubertal mice for use in adult mice. We found that adult animals were less sensitive than pubertal mice to estrogen, requiring higher levels to induce both stromal and epithelial proliferation. We are presently assessing the estrogen responsiveness in parous and nulliparous glands.

216

FUNCTION OF LRH-1 IN BREAST CANCER

K. A. Lazarus[1,3], A. L. Chand[1,2], C. D. Clyne[1,2]

[1]Cancer Drug Discovery, Prince Henrys Institute, Clayton, VIC, Australia
[2]Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia
[3]Life and Social Sciences, Swinburne University of Technology, Hawthorn, VIC, Australia

Background: Liver receptor homolog-1 (LRH-1; NR5A2; FTF) is a member of the Ftz-F1 family of nuclear receptors. LRH-1 belongs to the NR5A subfamily and has been linked to a number of functions ranging from development and cholesterol homeostasis to disease and proliferative processes [1] . LRH-1 is directly implicated in breast cancer progression via the regulation of local oestrogens and itself being regulated by the oestrogen receptor (ER) [2] . LRH-1 is aberrantly expressed in 50 % of invasive ductal carcinomas [3] . Our lab has previously shown that LRH-1 induces breast cancer cell proliferation, motility and invasion and regulates genes involved in epithelial mesenchymal transition and metastasis. In order to study the function of LRH-1 in breast cancer, we investigated its presence in MCF7 (ER^{+ve}) and MDA231 (ER^{-ve}) cell lines. Methods: We performed realtime quantitative PCR and western blots for LRH-1 detection. Western blots were performed using sc 5997 (N-terminus) and sc 5995 (C-terminus) specific antibodies (Santa Cruz). Results: LRH-1 mRNA levels were 20-fold higher ($p<0.05$) in MCF7 cell line when compared to MDA231. However in the two cell lines LRH-1 protein was readily detectable. There are three known isoforms of LRH-1, including hLRH-1v1 (61kDa), hLRH-1 (56kDa) and hLRH-1v2 (32kDa) that arise from alternative splicing and differ in truncations in the N-terminus. The hLRH-1 isoform is the most abundant and hLRH-1v2 is unable to activate transcription. Using the N-terminus specific antibody we found higher amounts of all the three isoforms in ER-ve cells. Using the C-terminus specific antibody we found varying levels of protein isoforms between the cell lines. These results suggest differential LRH-1 post-translational modification in these two cell lines. We are currently investigating post transcriptional regulation of LRH-1 and characterising its activity in a panel of ER+/- breast cancer cell lines. LRH-1 is therefore a potential novel prognostic and/or therapeutic target, which may lead to new treatments for breast cancer

(1) Fayard, E., J. Auwerx, and K. Schoonjans, LRH-1: an orphan nuclear receptor involved in development, metabolism and steroidogenesis. Trends in Cell Biology, 2004. 14(5): p. 250-260.

(2) Annicotte, J.-S., et al., The nuclear receptor liver receptor homolog-1 is an estrogen receptor target gene. Oncogene, 2005. 24(55): p. 8167-8175.

(3) Miki, Y., et al., Immunolocalization of liver receptor homologue-1 (LRH-1) in human breast carcinoma: Possible regulator of in situ steroidogenesis. Cancer Letters, 2006. 244(1): p. 24-33.

217

STROMAL ANDROGEN RECEPTOR-MEDIATED PARACRINE SIGNALING ENHANCES THE EFFICIENCY OF XENOGRAFTING HUMAN LOCALISED PROSTATE CANCER

R. Toivanen[1], D. Berman[2], H. Wang[1], M. Frydenberg[1], J. Pederson[3], R. Taylor[1], G. Risbridger[1]

[1]Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia
[2]Department of Pathology, The John Hopkins University School of Medicine, Baltimore, Maryland, United States
[3]TissuPath, Hawthorn, VIC, Australia

INTRODUCTION: Prostate cancer (PCa) research has been hindered by the inability to reliably xenograft localised human prostate cancer. Previous studies have demonstrated survival of these tumours *in vivo*, however the host mouse environment fails to facilitate active growth and proliferation of human prostate tissues, significantly reducing the utility of this model. This may be due to a lack of stromal androgen receptor (AR), which is down-regulated in human PCa, resulting in dampened stromal-cancer cell paracrine signalling. We hypothesised that providing an AR-rich stromal niche to localised PCa xenografts may enhance survival and growth of human cancer cells in mice due to a restoration of this paracrine signalling

METHODS: Localised PCa specimens were grafted sub-renally, with or without embryonic mouse prostatic mesenchyme into adult NOD-SCID male host mice supplemented with testosterone.

RESULTS: The incidence of tumour survival in xenografts was increased in the presence of inductive stroma (6/7 patients, 59% total inoculations) compared to xenografts alone (4/7 patients, 40% total inoculations). Compared to tumours grafted alone, xenografts with inductive stroma displayed increased tumour size, significantly higher tumour cell proliferation (indicated by ki67 quantification; 10.05±2.14% compared to 4.80±2.32%), and increased vasculature (identified by CD34 immunolocalisation). Importantly, the Gleason Grade of the original patient specimen was maintained in the xenografted tissue.

CONCLUSIONS: This is the first study to demonstrate actively proliferating xenografts of localised human PCa *in vivo*. Restoration of active stromal AR-mediated paracrine signalling significantly increased the efficiency and reliability of the model system. This significant advance will lead to a greater understanding of the factors that influence PCa progression, and could be utilised as a translatable pre-clinical model or allow personalised medicine for men with PCa.

218

TARGETING CASTRATE RESISTANT PROSTATE CANCER TUMOURS WITH ESTROGEN THERAPY

S. J. McPherson[1], S. Hussain[1], P. Balanathan[1], S. L. Hedwards[1], B. Niranjan[1], M. Grant[1], U. P. Chandrasiri[1], Y. Wang[2,3], R. A. Taylor[1], G. P. Risbridger[1]

[1]Prostate and Breast Cancer Research Group, Monash University, Anatomy & Developmental Biology, Clayton, Australia
[2]Department of Urologic Sciences, The Vancouver Prostate Centre, UBC, British Columbia, Canada
[3]Department of Cancer Endocrinology, BC Cancer Agency, Vancouver, Britisth Columbia, Canada

Prostate cancer (PC) is the most common malignant disease in aging men requiring androgens for growth and differentiation. Androgen ablation therapy causes a temporary reduction in PC tumour burden but unfortunately PC begin to grow again in the absence of androgens to form castrate resistant PC (CRPC). P rogression to CRPC remains the main obstacle to the management of advanced PC and new therapeutic strategies must be created to target this incurable stage of disease. Selective estrogen receptor modulators (SERMs) are a class of drugs with mixed estrogen agonistic/ antagonistic activity that holds promise in fulfilling this need.

This study reports the effect, the cellular targets and mechanism of action of a βSERM (ER β agonist, 8β-VE2) on prostate tissues and PC cells. Mechanistically different to castration, we show the beneficial pro-apoptotic actions of ERβ stimulation by 8β-VE2 in PC. In mouse models, 8β-VE2 induces extrinsic apoptosis in prostatic stromal, luminal and castrate-resistant basal epithelial cells, via activation of TNFα signalling. In human PC, 8β-VE2 causes apoptosis in Gleason Grade 7 xenografted PC tissues and androgen-independent cell lines (PC3 and DU145) via caspase-8. We also show specific difference in the expression of tumourigenic factors, VEGF-A and VEGF-C protein post-8β-VE2 treatment. VEGF-A was significantly decreased in 8β-VE2 treated DU145 and VEGF-C was significantly decreased in 8β-VE2 treated PC3 cell lines *in vitro*. The clinical significance of 8β-VE2 was evident by an increase in PC3 tumour doubling time (~2 fold) following 8β-VE2 treatment concurrent with a significant increase in apoptosis and reduction in proliferation.

These data provide the first evidence of that ERβ-induced apoptosis in PC tissues targets androgen-independent (and dependent) cells. Consequently, these data establish the rationale for the potential use of ER-specific modulators as new options for the treatment of CRPC.

(1) McPherson, SJ et al (2010) PNAS 107(7):3123-8

219

INVASIVE BEHAVIOURS IN HUMAN GRANULOSA CELL TUMOURS ARE ASSOCIATED WITH LOSS OF BETAGLYCAN AND THE DOWNREGULATION OF *MMP-15* AND *MMP-16* MRNAS

M. Bilandzic, Y. Wang, J. K. Findlay, K. L. Stenvers

Prince Henry's Institute, Clayton, VIC, Australia

Matrix metalloproteinases (MMPs) degrade components of the extracellular matrix and are essential regulators of tissue remodeling. Expression of specific subsets of MMPs influences both the proliferative behaviour and metastatic potential of cancer cells. Human ovarian granulosa cell tumours (GCTs) exhibit lower betaglycan gene expression compared to normal premenopausal ovary and overexpression of this receptor in GCT cells blocks their capacity to migrate and invade (1). However, t he mechanisms by which betaglycan regulates these cell behaviours are currently unknown. We hypothesised that betaglycan inhibits GCT cell invasion via the regulation of MMP expression and/or activation. By quantitative real-time PCR, GCTs (n = 17) exhibited a significant increase in mean *MMP-15* and *MMP-16* mRNA levels (6- and 2.5-fold, respectively; $p<0.05$) as compared to the normal ovary (n = 11), with no significant changes in the expression levels of *MMP-2, -3, -7, -9,* or *-14*. S table transfection of wildtype betaglycan into two GCT cell lines, KGN and COV434, resulted in significant decreases in the basal expression levels of *MMP-15* and *MMP-16* mRNAs ($p<0.001$). Both MMP-15 and MMP-16 are membrane-type MMPs known to activate pro-MMP2 and enhance cancer progression and invasion through tissue barriers (2). Gelatin zymography demonstrated a complete block in MMP-2 activity in the betaglycan-expressing GCT cells. Collectively, o ur data suggest that loss of betaglycan promotes a motile, invasive phenotype in GCT cells which is associated with aberrantly high *MMP-15* and *MMP-16* expression levels and a corresponding increase in MMP2 activation. Supported by the NHMRC of Australia (RegKeys 494802; 441101; 388904) and Victorian Government Infrastructure funds.

(1) 1. Bilandzic et al., 2009, Mol Endocrinol 23(4): 539-548
(2) 2. Hotary et al., 2000, J Cell Biol 49(6): 1309-23

220

THE FOXL2 C134W MUTATION IS PATHOGNOMONIC FOR ADULT GRANULOSA CELL TUMOURS OF THE OVARY

S. Jamieson[1,2], M. Alexiadis[1], P. J. Fuller[1,2]

[1]Prince Henry's Institute of Medical Research, Clayton, VIC, Australia
[2]Department of Medicine, Monash University, Clayton, VIC, Australia

Granulosa cell tumours of the ovary (GCT) represent ~5% of malignant ovarian cancers. Two distinct subtypes have been described based on clinical presentation and histological characteristics, the juvenile and the adult form. It has recently been reported that 97% of adult GCT carry a unique somatic mutation in the FOXL2 gene[1]. In this study we sought to confirm the presence of the FOXL2 Cys134Trp mutation in a geographically independent cohort of GCT and to examine the expression pattern of FOXL2 in these tumours. A total of 19 tumours with the histological diagnosis of adult GCT were examined for the presence of the mutation using direct sequence analysis. Two GCT-derived cell lines, COV434 and KGN, three juvenile GCT and 11 normal ovarian control tissues were also examined. Expression of the FOXL2 gene was determined using quantitative RT-PCR. 17 of the 19 adult GCT were found to harbour the mutation, of which, one was hemi/homozygous. Of the two cases with wild-type FOXL2 sequence, reappraisal suggests that they may have been misclassified. All three juvenile GCT and 11 normal ovaries were mutation negative. The presence of the mutation in the KGN cell line and absence in the COV434 cell line suggests they may be useful tools to study adult and juvenile GCT respectively. Expression levels were similar across the adult GCT and the normal ovary controls; one mutation negative GCT had high FOXL2 mRNA levels whereas the COV434 cells and two juvenile GCT lacked expression of FOXL2. This study provides confirmation that the presence of the FOXL2 C134W mutation is an almost universal feature of adult GCT and demonstrates that the mutation is not associated with altered FOXL2 expression. Despite many clinical and molecular similarities, juvenile GCT lack the mutation and therefore clearly have a distinct molecular aetiology. This unique FOXL2 mutation appears to be pathognomonic for adult GCT.

(1) Shah SP, et al. N Engl J Med. 2009; 360:2719-29

221

IN UTERO EXPOSURE TO A HIGH FAT DIET IS ASSOCIATED WITH AN INCREASED INCIDENCE OF PROSTATE ABNORMALITIES AND MIRNA EXPRESSION CHANGES IN ADULT RAT OFFSPRING

K. Chiam[1], N. Ryan[1], S. Jindal[1], S. Moretta[2], M. De Blasio[2], H. Harryanto[2], K. Kind[2], W. Tilley[1], J. Owens[2], T. Bianco-Miotto[1]

[1]Dame Roma Mitchell Cancer Research Laboratories, University of Adelaide and Hanson Institute, Adelaide, SA, Australia
[2]Discipline of Obstetrics and Gynaecology, School of Paedetrics and Reproductive, Research Centre for Early Origins of Health and Diseases, University of Adelaide, Adelaide, SA, Australia

An *in utero* exposure to a high fat diet, or a maternal high fat diet (MHFD), results in an excess nutritional supply to the foetus and has been associated with an increased incidence of breast cancer in rodent studies. While evidence from human epidemiological studies suggest that an excess foetal nutritional supply, as indicated by a high birth weight, is associated with an increased risk and mortality of prostate cancer, no studies have directly investigated if a MHFD is a risk factor for prostate cancer in adult offspring. Furthermore, the molecular basis for an association between a MHFD and increased susceptibility to cancer in adult offspring has been proposed to be due to epigenetic modifications, but this has yet to be demonstrated.

Using a rodent model, we demonstrated that a MHFD altered prostate development and was associated with an increased incidence of inflammation and hyperplasia in young adult offspring's prostates. *Gstp1*, which is hypermethylated and silenced in human prostate cancer, was significantly decreased in the prostates of offspring exposed to a MHFD compared to controls. Most importantly, we provide evidence that a MHFD is associated with epigenetic alterations that may contribute to the perturbed prostate development in the offspring. The DNA methyltransferase *Dnmt1* was decreased in the offspring prostates from the MHFD group compared to controls. Using a miRNA microarray analysis, we identified critical miRNAs (i.e miR-145 and miR-199a-5p) previously reported to be altered in human prostate cancer, to be overexpressed in offspring exposed to a MHFD. The common predicted network functions of these significantly altered miRNAs included cancer, cell death and proliferation. Common predicted target genes of these significantly altered miRNAs were identified and included CTCF, which is important in regulating the imprinted genes H19/ IGF2, and has been shown to be mutated and epigenetically silenced in prostate cancer.

222

DIET-INDUCED OBESITY ALTERS THE CENTRAL ACTIONS OF GHRELIN

D. I. Briggs, M. B. Lemus, Z. B. Andrews

Physiology, Monash University, Clayton, VIC, Australia

Circulating ghrelin is decreased in obesity and peripheral ghrelin is unable to induce food intake in obese mice. In the current study, we investigated whether ghrelin resistance was a centrally mediated phenomenon involving dysregulated NPY and AgRP circuits. We show that diet-induced obesity (DIO; 12 weeks) suppresses the neuroendocrine ghrelin system by decreasing acylated and total plasma ghrelin, decreasing ghrelin and GOAT mRNA in the stomach and decreasing expression of hypothalamic GHSR mRNA. Peripheral (i.p.) or central

(i.c.v.) ghrelin injection was able to induce food intake and arcuate nucleus Fos-immunoreactivity in chow-fed but not high fat-fed mice. DIO decreased expression of NPY and AgRP mRNA and central ghrelin was unable to promote expression of these genes. Injection of NPY i.c.v increased food intake in both chow-fed and high fat-fed mice, indicating downstream NPY/AgRP neural targets are intact and that defective NPY/AgRP function is a primary cause of ghrelin resistance in appetite-regulating pathways. Ghrelin resistance in DIO is not confined to the NPY/AgRP neurons, as ghrelin did not stimulate growth hormone secretion in DIO mice. Collectively, our data suggests that DIO causes ghrelin resistance by reducing NPY/AgRP responsiveness to plasma ghrelin and suppressing the neuroendocrine ghrelin axis to limit further food intake and weight gain. Ghrelin has a number of functions in the brain aside from appetite control including promoting cognitive function and mood regulation and protecting against neurodegenerative diseases. Thus, central ghrelin resistance may potentiate obesity-related cognitive decline, and restoring ghrelin sensitivity may provide therapeutic outcomes for maintaining healthy aging.

223

SUPPRESSOR OF CYTOKINE SIGNALLING 3 (SOCS3) IS PARTIALLY RESPONSIBLE FOR HIGH FAT DIET-INDUCED INFERTILITY THROUGH SUPPRESSION OF THE PREOVULATORY LH SURGE

H. J.L. McEwen[1,2], D. R. Grattan[1,2], G. M. Anderson[1,2]

[1]Centre for Neuroendocrinology, University of Otago, Dunedin, Otago, New Zealand
[2]Dept. of Anatomy and Structural Biology, University of Otago, Dunedin, Otago, New Zealand

Leptin is a critical neuroendocrine hormone for maintenance of fertility, whereby too little can result in infertility. Interestingly, too much of this hormone can also cause infertility, due to the development of central leptin resistance. Leptin may act in the anteroventral-periventricular nucleus (AVPV) and ventral-premammillary nucleus (PMV) regions to modulate the LH surge and tonic fertility respectively (1)(2). Exposure to leptin induces leptin resistance by mechanisms such as upregulation of SOCS3, an inhibitor of leptin signaling (3). Previously we have shown that forebrain-specific SOCS3-KO females on a high-fat-diet (23% fat) (HFD) showed resistance to obesity-induced infertility compared to control mice on the same diet. To gain a better understanding of the mechanisms involved in this infertility, transgenic mice were generated using DBA/2J mice with Cre/loxP-mediated forebrain neuronal SOCS3 deletion (SOCS3-KO). SOCS3-KO and control littermates were fed a HFD or a standard chow diet (<5% fat). These mice were subjected to an exogenous estradiol-induced preovulatory surge and a leptin challenge to determine hypothalamic leptin sensitivity (indicated by activation of pSTAT3). LH surges were detected in 5/5 SOCS3-KO but only 2/5 control HFD mice. SOCS3-KO and control mice fed a HFD or standard chow diet for over 130 days were injected with leptin and brain tissue collected after 4 hours. AVPV and PMV brain regions were observed for pSTAT3-positive cells using immunohistochemistry. The AVPV showed signs of leptin resistance in the HFD groups (>86% reduction in pSTAT3 immunoreactive cells, $p<0.05$), however no signs of resistance were detected in the PMV. These data support the hypothesis that SOCS3 upregulation in the AVPV is a contributing factor to HFD-induced infertility preventing a preovulatory LH surge. Other contributing factors are likely to also be involved in this HFD-induced infertility, since we have previously shown that even SOCS3-KO mice eventually become infertile with prolonged HFD feeding.

(1) Quennell J.H. et. al. Leptin indirectly regulates gonadotropin-releasing hormone neuronal function. Endocrinology 150:2805-2812.2009.
(2) Donato J. et. al. The ventral premammillary nucleus links fasting-induced changes in leptin levels and coordinated luteinizing hormone secretion. J Neurosci 29:5240-5250.2009.
(3) Tups A. Physiological models of leptin resistance. J Neuroendocrinol 21:961-971.2009.

224

GLUCOCORTICOID REGULATION OF LIPOLYSIS GENES AND THE ROLE OF MINERALOCORTICOID RECEPTORS DURING ADIPOGENESIS

I. Lys[1], J. Boon[3], S. Wong[4], J. Ng[2], T. J. Cole[2]

[1]Institute of Food, Nutrition and Human Health, Massey University, Wellington, New Zealand
[2]Department of Biochemistry and Molecular Biology, Monash University, Melbourne, VIC, Australia
[3]Department of Physiology, Monash University, Melbourne, VIC, Australia
[4]Molecular Pathology, Peter MacCallum Cancer Centre, Melbourne, VIC, Australia

Obesity is a serious health problem in the Western world. Steroid hormones such as the glucocorticoid cortisol are proposed to mediate adipose development in obesity and help maintain metabolic homeostasis. Glucocorticoid treatment and hypercortisolemia can cause distinct changes in the adiposity of individuals indicating that glucocorticoids play a critical role in regulating adipocyte differentiation, adipose tissue accumulation and lipolysis . Glucocorticoids can bind to both glucocorticoid receptors (GR) and mineralocorticoid receptors (MR) in adipocytes and contribute to the process of adipogenesis. This study utilized a mouse model lacking functional GR, an essential mediator for glucocorticoid signalling, to investigate the role of glucocorticoid signalling via the MR and GR during adipogenesis.

Mouse embryonic fibroblasts derived from E15 fetal GR-deficient and wildtype normal (WT) mice were cultured over a two week period, with and without eplerenone (a specific MR antagonist) and differentiated into the adipocyte cell lineage. The resulting numbers of adipocyte-like cells were compared by quantification with Oil Red O staining. Preliminary results indicate that eplerenone treated and non-treated derived adipocyte cells isolated from GR-deficient mice contained significantly lower lipid levels and total adipocyte cell numbers when compared to those from WT mice. However there was no significant difference observed in total adipocyte cell numbers between eplerenone-treated and non-treated control cells from GR-deficient mice. Gene expression analysis was completed using quantitative real-time-PCR for several markers of lipolysis (hormone sensitive lipase, cyclic-nucleotide phosphodiesterase 3B and adipose triglyceride

lipase). There was a significant decrease in the mRNA levels of these lipolysis markers in adipocytes obtained from GR-deficient mice. In summary, these findings indicate glucocorticoid signalling via GR is an important pathway leading to adipocyte differentiation and may provide a potential receptor target for therapeutics against obesity.

225

ALPHA-MELANOCYTE STIMULATING HORMONE (AMSH) ACTS DIRECTLY ON MUSCLE TO INCREASE PUTATIVE THERMOGENESIS

B. A. Henry[1], M. A. Cowley[1], S. D. Clarke[1], B. Leury[2], I. J. Clarke[1]

[1]Dept of Physiology, Monash University, Clayton, VIC, Australia

[2]School of Land and Environment, University of Melbourne, Melbourne, VIC, Australia

Pro-opiomelanocortin encodes endorphins and melanocortins in the pituitary gland and the hypothalamus. Melanocortins, including αMSH, act centrally via the melanocortin-4 receptor to reduce food intake. The melanocortin-5 receptor is expressed in skeletal muscle but effects of αMSH on muscle tissue remain to be elucidated. We aimed to determine the effect of αMSH on skeletal muscle using an isolated hind-limb model in ovariectomised ewes. Animals (n=4-5/ group) were program-fed (1100-1600h) to establish a post-prandial thermogenic response in muscle (1). Cannulae were inserted into the femoral artery and vein and the jugular vein; arterial cannulae were used for infusion whereas venous cannulae were used for blood sampling. Plasma metabolites and levels of α MSH were measured in jugular and femoral venous samples. Temperature recording devices (dataloggers) were implanted into the muscle of the treatment limb. Temperature was recorded every 15min and αMSH was infused at doses of 0.1, 1, 5 and 10 μg/h for 9h with saline infusion as control. αMSH increased heat production in skeletal muscle of the treatment limb but did not alter food intake. High doses (5 and 10 μg/h) of αMSH increased temperature immediately upon infusion, whereas low doses (0.1 and 1.0 μg/h) increased post-prandial temperature only. Infusion of 5 and 10 μg/h αMSH increased both femoral and jugular levels of αMSH, whereas 0.1 μ g/h infusion increased αMSH levels in femoral vein plasma only. These data suggest that, at lower doses, αMSH increases heat production by direct action on skeletal muscle. As expected, plasma lactate and glucose levels increased after feeding, but non-esterified fatty acids levels were reduced, with no effect of αMSH treatment. We conclude that αMSH acts directly on muscle tissue to increase post-prandial thermogenesis, indicating a novel means for increasing energy expenditure.

(1) Henry et al (2008) Endo 149:2019-26

226

LOSS OF 1,25(OH)$_2$VITAMIN-D3 IN BETA-CELLS CAUSES IMPAIRED GLUCOSE TOLERANCE AND GLUCOSE-STIMULATED INSULIN SECRETION: METABOLIC EFFECTS OF 1-A-HYDROXYLASE (CYP27B1) KNOCKOUT IN INSULIN-PRODUCING CELLS.

S. L. Lau[1], R. Stokes[1], B. Ng[1], R. Clifton-Bligh[2], J. E. Gunton[1]

[1]Diabetes & Transcription Factors, Garvan Institute, Darlinghurst, NSW, Australia

[2]Cancer Genetics, Kolling Institute, St Leonards, NSW, Australia

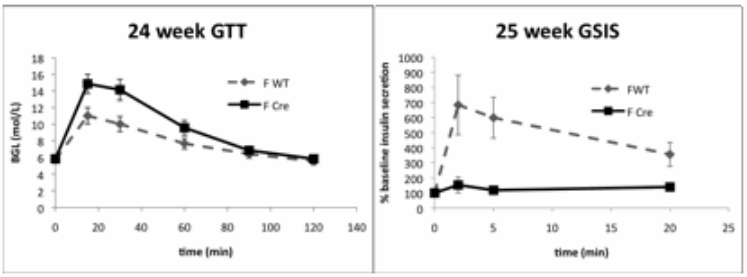
BACKGROUND: The Cyp27B1 gene encodes the enzyme 1α-hydroxylase, which converts 25OH-Vitamin-D to the active hormone 1,25(OH)2Vitamin-D3. We have found that 1α-hydroxylase is present in beta-cells but its role there is unknown.

METHODS: Floxed Cyp27B1 mice were bred with mice expressing RIP-Cre (Cre-recombinase driven by the rat insulin promoter) to generate β-cell Cyp27B1 knockout mice (β-Cyp27B1-KO). β-Cyp27B1-KO mice were compared to floxed-control littermates. In separate studies, 17-25 mice of each genotype and gender underwent glucose tolerance testing (GTT) and glucose-stimulated insulin secretion (GSIS) at either 7 weeks or 24 weeks. A subset of mice had insulin tolerance testing, DEXA scanning and food consumption studied at weekly intervals following GSIS. At sacrifice, organs were weighed.

RESULTS: Female β-Cyp27B1-KO mice had significantly worse glucose tolerance than floxed-control littermates at both 7 and 24 weeks (Figure). Male β-Cyp27B1-KOs showed a similar trend, with significantly higher glucoses at 30 minutes. At both 7 and 24 weeks (Figure), female β-Cyp27B1-KO had lost first-phase insulin secretion, with similar findings in males by 24 weeks.

There were no significant differences in insulin sensitivity or food consumption. β-Cyp27B1-KOs, however, had significantly lower bone mineral content (27wk females 0.26 vs 0.33g, $p<0.001$) and greater body fat-% (19.4% vs 13.3%, $p<0.0001$). At sacrifice, body weight did not differ, but consistent with DEXA results, β-Cyp27B1-KOs had greater subcutaneous and epididymal fat masses (10wk females: epididymal fat 1.6% vs 0.8% of body weight, $p<0.0001$).

CONCLUSIONS: These results suggest that β-Cyp27B1-deletion in beta-cells adversely effects first-phase insulin secretion and glucose tolerance.The unexpected alterations in BMC and fat mass were interesting and could be secondary to the beta-cell defect, an effect of deletion in the insulin-expressing subset of hypothalamic neurons, and/or related to the RIP-Cre.



227

KISSPEPTIN IS ESSENTIAL FOR THE FULL PREOVULATORY LH SURGE IN SHEEP

K. S. Yap[1], M. Shahab[2], R. P. Millar[3], I. J. Clarke[1], J. T. Smith[1]

[1]Department of Physiology, Monash University, Melbourne, VIC, Australia
[2]Department of Animal Sciences, Qauid-I-Azam University, Islamabad, Pakistan
[3]Medical Research Council Human Reproductive Sciences Unit, The Queens Medical Research Institute, Edinburgh, United Kingdom

Kisspeptin is the product of the Kiss1 gene, binds to the receptor GPR54, and stimulates gonadotrophin-releasing hormone (GnRH) secretion. Kisspeptin/GPR54 signalling is critical for puberty and normal reproduction. In sheep, Kiss1 mRNA expressing cells are found in the arcuate nucleus and dorsal preoptic area and both appear to mediate the positive feedback effect of estradiol to generate the preovulatory GnRH/luteinizing hormone (LH) surge. To determine the role of kisspeptin on the surge, we administered the kisspeptin antagonist p234-penetratin (8 h continuous intracerebroventricular infusion, 300 µg/h; initial 200 µg loading dose) or vehicle control to ewes. Prior to infusions, all ewes were subjected to an estradiol benzoate (EB, 50 µg intramuscular) induced LH surge. In all control ewes, EB resulted in LH surges (n=6). Kisspeptin antagonist treatment significantly inhibited LH surges in ewes (area under the LH curve analysis revealed LH surges were 56% lower in kisspeptin antagonist treated animals, $P<0.05$, n=6), and completely prevented the surge in 2 animals. To further determine the role of kisspeptin on the LH surge, we examined whether the response to kisspeptin treatment (50 µg iv) varies between the luteal phase of the estrous cycle and the late-follicular phase, just prior to the LH surge (n=5-6 per group). Kisspeptin significantly stimulated GnRH and LH in all animals compared to vehicle treated controls. The LH response to kisspeptin was greater ($P<0.05$) in ewes during the late-follicular phase. However, the GnRH response to kisspeptin appeared unchanged prior to the surge. These data suggest the GnRH response to kisspeptin (kisspeptin potency) does not change prior to the LH surge. Estrogen priming of the pituitary gland is the most probable explanation for the increased LH response. Despite this, kisspeptin does appear to play an essential role in receiving estrogen stimulatory signals and generating the positive feedback GnRH/LH surge.

228

SEX-SPECIFIC REGULATION OF GLUCOCORTICOID EXPOSURE IN PRETERM PREGNANCIES BY P-GP AND MRP-1

N. A. Hodyl, M. J. Stark, V. L. Clifton

Robinson Institute, University of Adelaide, Elizabeth Vale, SA, Australia

Fetal glucocorticoid exposure is tightly regulated across gestation by the placental glucocorticoid barrier. This is comprised in part by the cortisol inactivating enzyme 11beta hydroxysteroid dehydrogenase (11βHSD2) together with multidrug resistant t ransmembrane efflux proteins. Two such proteins are P-glycoprotein (P-gp) and multidrug resistance protein-1 (MRP-1), which are located on opposite surfaces of the syncytiotrophoblast. Observations of a greater prophylactic effect of the synthetic glucocorticoid betamethasone in female preterm births compared to males have been demonstrated. We questioned whether this was due to sex-specific alterations in placental P-gp and MRP-1 expression. The aim of this study was to assess placental P-gp and MDR-1 expression, together with 11βHSD2 activity in placenta from preterm pregnancies.

Methods: Placental samples were collected from women who delivered preterm (24-36 weeks n=42) or at term (n=11). P-gp and MRP-1 mRNA was measured by qRT-PCR and 11βHSD2 activity by radiometric conversion assay. Betamethasone exposure was classified as delivery <72 or >72 hours after maternal steroid administration.

Results: Placental P-gp expression increased exponentially with advancing gestation in the preterm infants (R^2=.364, p=0.018), but expression returned to low levels at term. P-gp expression was inversely correlated with 11βHSD2 activity in females (r=-.664, p=0.018) but not in males. MRP-1 expression decreased over gestation in female preterm placenta (r=-0.547, p=0.02) but not males. Neither P-gp nor MRP-1 was affected betamethasone exposure.

Conclusion: Antenatal betamethasone does not alter expression of P-gp or MRP-1 in preterm placenta. However, P-gp and MRP-1 appear to play a strong role in maintaining fetal glucocorticoid homeostasis across gestation. This is particularly evident with a female preterm infant, where our results suggest increased glucocorticoid efflux from the placenta by P-gp when 11βHSD2 activity is at its lowest and increased glucocorticoid influx by MRP-1 in early preterm deliveries. This study supports previous observations of greater placental sensitivity and adaptation in the female preterm neonate compared to male.

229

IUGR INDUCED UPREGULATION OF PGHS-1 CONTRIBUTES TO INCREASED RISK OF PRETERM BIRTH

H. K. Palliser[1,2], T. Zakar[1,3], I. M. Symonds[1,3], J. J. Hirst[1,2]

[1]Mothers & Babies Research Centre, University of Newcastle, Newcastle, NSW, Australia
[2]School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, NSW, Australia
[3]School of Medicine and Public Health, University of Newcastle, Newcastle, NSW, Australia

Intrauterine growth restriction (IUGR) is a known risk factor of spontaneous preterm birth however the mechanisms of the relationship remain unknown. Prostaglandins are key regulators of the labour pathway and their synthesis is regulated by the enzyme Prostaglandin H Synthase (PGHS). We have established a model of IUGR in the guinea pig which results in a slight but significantly earlier gestational age at delivery. We hypothesised that pregnancies associated with IUGR would exhibit preterm upregulation of intrauterine PGHS-1 protein expression and increased vulnerability to preterm birth. Pregnant guinea pigs underwent surgery to induce IUGR at mid gestation and myometrium, placenta and amnion were collected throughout late gestation and at labour. PGHS-1 protein expression was measured by western blot. IUGR associated pregnancies (as assessed by fetal brain to liver weight ratios) delivered significantly earlier than sham operated (control) pregnancies (GA69±0.2 and GA71±0.5 respectively, $P<0.05$). Myometrial PGHS-1 expression was significantly increased in IUGR pregnancies at late gestation ($P<0.05$) with no difference found at labour. Myometrial PGHS-1 expression in the non gravid horn was significantly lower than gravid myometrial PGHS-1 expression in late gestation and at labour ($P<0.05$). Amniotic PGHS-1 expression significantly increased over late gestation and interestingly, this occurred earlier in IUGR pregnancies (by 65 days gestation) than in controls (68 days). Placental PGHS-1 significantly increased with gestation and labour ($P<0.05$) however unlike the myometrium and amnion, no effect of IUGR was found. These results support the role of PGHS-1 in prostaglandin synthesis in preterm and term labour. In addition, these studies suggest IUGR markedly upregulates PGHS-1 expression in IUGR pregnancies in late gestation, increasing their susceptibility to preterm birth.

230

EXPRESSION OF HUMAN LEUKOCYTE ANTIGEN BY MONOCYTE SUBSETS DURING PREGNANCY

A. Osei-Kumah[1], D. Krumbiegel[2], I. Nicholson[2], R. Grose[2], N. A. Hodyl[1], M. J. Stark[1], H. Zola[2], V. L. Clifton[1]

[1]Obstetrics & Gynaecology, Robinson Institute, University of Adelaide, Adelaide, SA, Australia
[2]Leukocyte Biology Laboratory, Women's and Children's Health Research Institute, Adelaide, SA, Australia

Introduction: During pregnancy, we have shown that the asthma exacerbation rate is high, increasing the risk of an adverse neonatal outcome, including intrauterine growth restriction, preterm delivery and still birth. In a majority of cases exacerbations are un-resolvable with inhaled glucocorticoid treatment, suggesting pregnancy changes the asthma phenotype to a form that is non-responsive to asthma treatment. This may be related to maternal adaptations in immune pathways that occur with pregnancy. In normal pregnancy, maternal circulating leukocytes undergo modifications in cell concentration, phenotype and function over the course of pregnancy. However little is known about how this adaptation in pregnancy is influenced by the presence of maternal asthma. We aim to characterise leukocyte sub-populations and phenotypes in blood collected from pregnant non-asthmatic and asthmatic women.

Hypothesis: We propose that maternal asthma worsens during pregnancy due to altered leukocyte phenotypes, including increased monocyte $CD14^{dim}CD16^{+}$ subset, changes in markers of activation such as human leukocyte antigen (HLA)-DR and disturbances in T cell subsets particularly Tregs, Th1 and Th2.

Methods: Venous blood was collected from pregnant asthmatic subjects (n =10) and controls (n =10) at 12, 18 and 30 weeks gestation. Peripheral blood mononuclear cells (PMBCs) were isolated at all three time points. Multi-parameter flow cytometry analysis using appropriate antibody pairs was used to determine T cell and monocyte subsets.

Results: Our preliminary analysis demonstrated that there were no differences in T cell subtypes in control and asthma group as pregnancy progressed. However there were differences in monocyte subsets with differential expression of activation marker HLA-DR in the asthmatic group. Additionally, a subset of subjects had higher percentage of monocytes expressing HLA-DR. The expression of 'pro-inflammatory' monocyte phenotype $CD14^{dim}CD16^{+}$ was also identified in some asthmatic subjects.

Discussion: Circulating monocytes are heterogeneous in phenotype and function. $CD14^{dim}$ CD16+ phenotype expands during infection and inflammatory response. HLA-DR is a cell surface molecule that mediates antigen processing for effective immune responses. The differential expression of leukocyte subsets and activation states in pregnancies complicated by asthma may be part of the mechanism contributing to worsening asthma during pregnancy.

231

EVALUATION OF NEUROENDOCRINE TUMOURS USING SOMATOSTATIN RECEPTOR PET

J. Hoang[1,2], M. L. Sam[1], V. Kumar[1], D. Boddeti[1], S. Angelides[1]

[1]Department of Nuclear Medicine, PET and Ultrasound, Westmead Hospital, Westmead, NSW, Australia
[2]Department of Endocrinology, Gosford Hospital, Gosford, NSW, Australia

Introduction:

Somatostatin receptor scintigraphy (Octreoscan) is an established imaging modality in the initial and post-treatment evaluation of neuroendocrine tumours (1,2). Positron emission tomography (PET) has been a major advancement in oncology imaging. The recent

literature of PET using Gallium-68 labelled Octreotide in the evaluation of neuroendocrine tumours reports detection rates superior to that of Octreoscan in both early and metastatic disease (3,4). What is less clear is the prognostic value and impact on patient management associated with this new imaging modality.

Method and Results:

Between February 2009 and January 2010, 71 patients with suspected or confirmed neuroendocrine tumours were prospectively recruited for Gallium-68 Octreotide (GaO) PET at a single centre. 75 scans were performed. Clinical, radiological and biochemical data were collected.

GaO PET was positive in all of the 41 histologically confirmed cases of neuroendocrine tumours across a wide range of histology and in both limited and metastatic disease. Of 8 patients with histologically confirmed disease referred for localization of the primary lesion, 4 (50%) had positive PET findings. Lower standardized uptake value (SUVmax) was associated with less differentiated disease (p=0.002). There was no significant correlation between SUVmax and chromogranin A levels. GaO PET resulted in change of management in 24% of patients.

Conclusion: Somatostatin receptor PET using Gallium-68 labelled Octreotide can effectively detect neuroendocrine tumours and impact on treatment in a significant proportion of patients. The standardized uptake value as a measure of somatostatin receptor expression is positively correlated with the degree of tumour differentiation.

(1) Krenning EP, Kwekkeboom DJ, Bakker WH et al. Somatostatin receptor scintigraphy with [111In-DTPA-D-Phe1]- and [123I-Tyr3]-octreotide: the Rotterdam experience with more than 1000 patients. Eur J Nu

(2) Krausz Y, Keidar Z, Kogan I et al. SPECT/CT hybrid imaging with In111-pentetreotide in assessment of neuroendocrine tumours. Clinical Endocrinol 2003; 59: 565-73

(3) Kowalski J, Henze M, Schuhmacher J et al. Evaluation of positron emission tomography imaging using (68Ga)-DOTA-DPhe1-Tyr3-octreotide in comparison to (111-In)-DTPAOC SPECT: first results in patient

(4) Win Z, Rahman L, Khan S et al. Ga-68 DOTATATE PET imaging in neuroendocrine and neuroectodermal tumours. Nucl Med Commun 2007; 28: 359-363

232

TESTOSTERONE LEVELS PREDICT SEXUAL ACTIVITY IN OLDER MEN

Z. Hyde[1,2], L. Flicker[1,2], G. J. Hankey[2,3], O. P. Almeida[1,4,5], K. A. McCaul[1,2], P. Chubb[2,6], B. B. Yeap[2,7]

[1]Western Australian Centre for Health and Ageing, Centre for Medical Research, Western Australian Institute for Medical Research, Perth, WA, Australia

[2]School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia

[3]Department of Neurology, Royal Perth Hospital, Perth, WA, Australia

[4]School of Psychiatry and Clinical Neurosciences, University of Western Australia, Perth, WA, Australia

[5]Department of Psychiatry, Royal Perth Hospital, Perth, WA, Australia

[6]PathWest, Department of Biochemistry, Fremantle Hospital, Fremantle, WA, Australia

[7]Department of Endocrinology and Diabetes, Fremantle Hospital, Fremantle, WA, Australia

Background . Hypogonadism is associated with erectile dysfunction and loss of libido in young and middle-aged men, but factors limiting sexual activity in older men are less well understood. Decreased sexual activity is thought to result from medical comorbidity and social factors, or is viewed as an ‘inevitable' part of the ageing process. We aimed to determine the proportion of older men who are sexually active, and to explore the factors predictive of sexual activity in this age group.

Methods . The Health In Men Study (HIMS) is a population-based study of men living in Perth, Western Australia. Between 1996-99, 12,203 community-dwelling men aged 65 years and older were recruited via the electoral roll, and completed a questionnaire, providing a range of risk factor data. In 2001-04, 5,585 men underwent a second assessment, in which sera was obtained from 4,249. In 2008-09, sexual activity was assessed in a third survey of 3,274 men aged 75-95 years. Logistic regression was performed to explore factors associated with being sexually active in a cross-sectional model utilising the entire sample (n=3,274), and in a longitudinal model comprising only men who provided sera at the second assessment.

Results . Of the 2,783 men (85.0%) who provided data on sexual activity, 857 (30.8%; 95% confidence interval [CI] 29.1-32.5%) had sex at least once in the past 12 months. In cross-sectional analyses, increasing age, lack of interest in sex by the partner, physical limitations of the partner, osteoporosis, prostate cancer, diabetes, anti-depressant use, and beta blocker use were independently associated with reduced odds of sexual activity. Cohabiting with a partner and being from a non-English speaking background were associated with increased odds. In a longitudinal model utilising sex hormone data, higher testosterone levels in 2001-04 were independently associated with increased odds of being sexually active at follow-up. Other factors were similar to those in the cross-sectional model.

Conclusions . One third of older men are sexually active. Social factors and medical comorbidity are major determinants of sexual activity in older men, but endogenous testosterone levels remain an independent predictor. Low testosterone levels appear to be a potentially modifiable risk factor for reduced sexual activity. Further investigation is required to determine if measures that maintain circulating testosterone levels can improve sexual activity in older men.

233

THE METABOLIC EFFECT OF A HIGHLY B2-SELECTIVE AGONIST, FORMOTEROL, IN HUMANS

P. Lee[1,2], R. O. Day[3], J. R. Greenfield[2], K. K.Y. Ho[1,2]

[1]Pituitary Research Unit, Garvan Institute of Medical Research, Sydney, NSW, Australia
[2]Department of Endocrinology, St Vincent's Hospital, Sydney, NSW, Australia
[3]Department of Clinical Pharmacology, St Vincent's Hospital, Sydney, NSW, Australia

Adipose tissue and muscle are major sites of whole body metabolism that are regulated by the sympathetic nervous system via β2-adrenoceptors (ARs). Activation of energy metabolism as a therapeutic approach for obesity has been hindered by limited specificity of available β2-agonists, which induce tachycardia. Formoterol is a new generation, highly β2-selective agonist, with less than 0.3% activity on β1-ARs. The metabolic effects of formoterol in humans have not been studied. Aim: To investigate the metabolic effects of formoterol. Method: We undertook a) a dose-finding study in 4 subjects, administered 80, 160 and 320μg daily of oral formoterol for 1 week each and b) a detailed metabolic evaluation in 12 subjects before and at the end of 1 week treatment. Energy expenditure (EE) and fat oxidation (Fox) were quantified by indirect calorimetry with diet-induced thermogenesis measured over 120 minutes after a standardised meal. Non-esterified free fatty acid (NEFA) was measured by chemiluminescence method. Statistical analysis was performed after log-transformation where appropriate. Results: In the dose finding study, 160 μg achieved maximal increase in resting EE and Fox without inducing tachycardia. In the metabolic study, this dose increased resting EE (11±2%) and basal Fox (23±4%) but not NEFA nor heart rate (HR) [Table]. Peak EE was enhanced during formoterol therapy by 10±3% following the standardised meal. Fox was suppressed during the meal with the nadir unaltered by formoterol. In summary, formoterol 160 μg/day increases resting energy expenditure, fat oxidation and post-prandial peak thermogenesis in humans, without inducing tachycardia. From this first metabolic evaluation of formoterol in humans, we conclude that formoterol may be useful in the management of obesity. (supported by NHMRC Australia)

	Basal				Post-prandial	
	HR (bpm)	NEFA (mM)	EE (kcal/d)	Fox (mg/min)	Peak EE (kcal/d)	Nadir Fox (mg/min)
Baseline	70±2	0.2±0.0	1450±49	53±4	1681±76	41±4
Formoterol	74±3	0.2±0.0	1611±54	66±5	1758±76	39±3
p-value	0.2	0.9	<0.001	<0.01	0.02	0.4

234

PHAEOCHROMOCYTOMA, BACK WITH A BITE - MANY FACES, MANY DILEMMAS

S. P. Joseph, K. Campbell, M. P. Doogue

Endocrinology, Flinders Medical Center, Adelaide, SA, Australia

Phaeochromocytomas are catecholamine producing neuroendocrine tumours with multiple manifestations. Biochemical assessment with metanephrines and chromogranin A is the mainstay of diagnosis and follow up. Malignant phaeochromocytoma has a poor prognosis and the optimal treatment remains uncertain. We report a case of phaeochromocytoma in a 57 year old woman, who presented with paralytic ileus and two years after apparent surgical cure developed metastatic disease.

Mrs. M presented in March 2008 with paralytic ileus and a CT pulmonary angiogram revealed a 9cm left adrenal mass. Phaeochromocytoma was confirmed biochemically: metanephrine 36,500pmol/L (<500pmol/L), normetanephrine 21,200pmol/L (<900pmol/L). After treatment with intravenous phentolamine, gastric function returned and she was stabilized on oral alpha and beta blockers prior to elective adrenalectomy. Serial plasma metanephrine measurement perioperatively found half-lives of metanephrine and normetanephrine to be 52 and 150 minutes respectively. After adrenalectomy her blood pressure was well controlled on candesartan alone and her diabetes resolved. Genetic testing revealed no mutations associated with phaeochromocytoma. Of note, 2 years prior, she had an episode of transient stress induced cardiomyopathy diagnosed as being secondary to perioperative intranasal adrenaline.

For 2 years she was well with normal biochemistry (metanephrine 170pmol/L, normetanephrine 560pmol/L, chromogranin A 9U/L (<17U/L). In March 2010 diabetes recurred and tumour markers were elevated: plasma metanephrine 3,890 pmol/l, normetanephrine 1,450pmol/l and chromogranin A 156U/L. The apparent doubling time of metanephrines after recurrence was 1-2 months. Imaging identified multiple metaiodobenzylguanidine (MIBG) avid skeletal and liver metastases . In May 2010 she was treated with I131- MIBG 3.9 GBq, with no evidence of consequent bone marrow suppression.

The half-life of metanephrines suggests the benefit of short periods of rest prior to sampling may be overstated. It is unknown whether MIBG avidity predicts treatment response. The role of newer chemotherapeutic agents in the treatment of phaeochromocytoma is not established.

235

ACROMEGALY – WHERE IS THE TUMOUR?

N. Nandi, V. Shenoy, D. M. Howard, S. Chitturi

Medicine, Royal darwin Hospital, Tiwi, NT, Australia

Acromegaly without identifiable pituitary mass is rare. The GH excess may result from ectopic GHRH production by neuroendocrine tumors or extra-pituitary production of GH itself. Literature is scant on prognosis and management of patients with Acromegaly caused by GHRH excess where the source remains unidentified.

A 67 year old indigenous lady was referred for management of diabetes and hypertension, currently on Metformin, Ramipril, Atenolol and Amlodipine. She was noted to have prognathism in previous records and had pituitary imaging in 2003, which was normal. She was otherwise well except for intermittent atrial fibrillation for which she was on Warfarin.

Examination revealed BP 140/80 mm Hg and clinical evidence of acromegaly with frontal bossing, prognathism, coarse facial features, spade like fingers, and increased heel pad thickness (30 mm on X-ray). Investigations revealed an elevated IGF-1 of 460 µg/ml (normal<360) and confirmed with an alternative lab as 50 nmol/L (normal 5-30), and non suppressible GH levels (> 5 mu/l) on a glucose suppression test. Chromogranin A was 23 U/L (<17.2), and platelet serotonin as well as IGFBP3 were normal. A dedicated MRI Pituitary scan (April 2009) was normal and showed no difference from the previous scan done in Nov 2003. Other anterior pituitary function tests were all normal.

An ectopic focus was investigated by CT scan of chest, abdomen and pelvis and an octreotide scan, both of which were normal. GHRH done from baseline (0m) sample of glucose suppression testing was very high at 199 ng/ml (Ref: 9.4 ± 5.5 ng/ml) . As she has high CV risk Octreotide was started to control GH excess. Six months post treatment, IGF-1 normalized (19nmol/L),the BP and BGLs remain well controlled. Even though medical therapy was successful in controlling GH secretion, the source of GHRH remained unidentified after extensive investigation.

This case report is of an unidentifiable ectopic source of GHRH causing acromegaly.

236

PET SCANCER

M. Pace, D. Topliss

Department of Endocrinology & Diabetes, The Alfred, Melbourne, VIC, Australia

Case 1: 46-year-old woman with PTC (diffuse sclerosing variant) with extensive local lymph node metastases. Following total thyroidectomy and ^{131}I-ablation, the whole body scan (WBS) showed thyroid remnant uptake only. Subsequent CT revealed small bilateral pulmonary nodules which did not appear FDG-avid on whole body PET scanning. However, a focus of intense FDG uptake in the sigmoid colon highly suspicious for a primary colonic neoplasm was incidentally identified. Colonoscopy localised a large, fungating mass in the sigmoid consistent with adenocarcinoma on biopsy. She underwent a high anterior resection with histopathology confirming moderately-differentiated adenocarcinoma (T3 N0 MX, Stage II).

Case 2: 63-year-old woman with Hürthle cell carcinoma diagnosed incidentally on CT performed for aneurismal screening. WBS after thyroidectomy and ^{131}I-ablation revealed neck uptake only. A follow-up diagnostic ^{131}I WBS showed no evidence of residual or recurrent disease in the neck or elsewhere. Given the nature of Hürthle cell carcinoma, whole body PET was organised which, while confirming no malignancy in the neck, demonstrated a focus within the right hemipelvis in a loop of bowel. Colonoscopy identified a single, pedunculated polyp in the sigmoid shown to be a low-grade tubular adenoma.

Case 3: 50-year-old man with multifocal PTC with lymph node involvement and capsular extension diagnosed on a staging PET scan performed following the excision of melanoma. He underwent a total thyroidectomy and ^{131}I-ablation with no evidence of ^{131}I-avid metastases on WBS. Despite maintenance on TSH-suppressive thyroxine, his unstimulated thyroglobulin remained detectable. A further therapeutic ^{131}I dose was administered with WBS demonstrating faint neck uptake only. Follow-up imaging confirms no recurrent or metastatic disease.

Clinical Questions

- Given the detection of incidental hypermetabolic lesions is likely to become more common with the increasing use of PET scanning in thyroid and other malignancies, how useful is PET in identifying second primary malignancies?
- What is the role of PET scanning in the management of thyroid cancer?
- What features can be used to differentiate benign from malignant thyroid lesions diagnosed on PET?
- Once an incidental focal thyroid lesion has been detected by PET, how should it be further evaluated?

237

NOT ENOUGH FAT: LIPODYSTROPHY & LEPTIN DEFICIENCY

G. Thomas

Austin Health, Heidelberg, VIC, Australia

A 42 year old woman was admitted in August 2008 with decompensated chronic liver disease. Her past history included Acquired Generalised Lipodystrophy, Chronic Liver Disease secondary to NASH, T1DM, SLE, Autoimmune Haemolytic Anaemia & Thrombocytopaenia & Depression. Clinical features were typical of generalised lipodystrophy.

The patient was readmitted 2 months later with spontaneous bacterial peritonitis and worsening liver function, and received a liver transplant during that admission. She had reduced insulin requirements post-transplant, but requirements gradually increased.

In June 2009 the patient commenced leptin therapy. Her appetite drastically reduced, and insulin requirements decreased rapidly. She continues on leptin replacement indefinitely with reduced appetite and reduced insulin requirements.

Points for Discussion

What is lipodystrophy and how is it associated with leptin deficiency?

How does leptin deficiency result in insulin resistance, hypertrigliceridemia, dianetes and liver disease?

How do we manage the effects of lipodystrophy and leptin deficiency?

What are the effects of leptin replacement in lipodystrophy?

This is a rare case of acquired generalised lipodystrophy associated with hyperphagia, T1DM and severe insulin resistance, hypertriglyceridemia and cirrhosis secondary to NASH.

I will present the case and will then briefly review the lipodystrophy syndromes (in particular acquired generalised lipodystrophy which is often associated with autoimmune disorders) and their association with leptin deficiency.

I will summarise the actions of leptin and its effect on insulin sensitivity and fatty acid oxidation. Leptin deficiency results in insulin resistance and through limiting fatty acid oxidation also results in lipotoxicity in various organs. I will summarise reported studies of the effect of leptin replacement in humans, in both the short and long term.

(1) Leptin Deficiency: Clinical Implications and Opportunities for Therapeutic Interventions. Bluher, S et al. Journal of Investigative Medicine. 57(7):784-788, October 2009

238

INTRA-UTERINE GROWTH RETARDATION (IUGR) DUE TO 11P15 LOSS OF METHYLATION: ENDOCRINE AND METABOLIC CONSEQUENCES

J. Wong

Diabetes and Endocrinology, Royal Children's Hospital, Melbourne, VIC, Australia

We present the case of a boy with Russell-Silver syndrome due to a loss of methylation of the H19 locus on 11p15,with short stature,poor catch up linear growth despite use of growth hormone, early puberty, partial gonadal failure complicated by metabolic syndrome.He was born at 34 weeks getsation at 1.16 kg (< 3rd centile) and failed to show catch up growth. Orchidopexy for testicular maldescent was performed at age 2. Growth hormone was given from age 5, with modest response < 1st centile to > 10 centile by age 8, then sudden increased height velocity. Initial endocrine consultation occured at 13 years, where height was 147 cm, BMI 90th centile, marked acne, gross acanthosis nigricans of neck and axillae, BP 151/97. He had adult virilisation but 2 ml testes bilaterally. Bone age was 17, with fused epiphyses. Review of past growth pattern demonstrated rapid growth acceleratiom from 8-12 years, consistent with early pubertal onset.Investigations confirmed hypertension on 24 hour monitoring, likely steato-hepatitis (ALT 61 IU/L, focal hepatic fatty infiltration on CT), fasting hypercholestrolaemia (6.9 mmol/L) and hypertriglyceridemia (16.1 mmol/L). Oral glucose tolerance confirmed type 2 diabetes mellitus with insulin resistance:glucose 5.1 mmol/L rising to 13.2 mmol/L with high fasting insulin levels: insulin 39.7 mU/L rising to 420 mU/L. Gonadotrophins were elevated: LH 15.9 IU/L, FSH 27.2 IU/L. Testosterone 7.3 nmol/L was normal for an adolescent boy. Discussion: This case highlights the serious nature and spectrum of possible IUGR consequences.Epigenetic programming is recognized to result in foetal and subsequent post-natal growth restriction leading to adult disorders including diabetes and metabolic syndrome. IUGR maybe a model for more generalised hormone resistance; the severity and multiplicity of which maybe dependent on the severity or aetiology of the growth restriction. The partial gonadal failure in our subject may reflect hormone resistance as a result of IUGR rather than a post surgical event.

239

EXPOSURE OF RATS TO A SIMULATED SHIFT WORK SCHEDULE DURING PREGNANCY IMPACTS THE HEALTH AND DEVELOPMENT OF THE OFFSPRING.

T. J. Varcoe, N. Wight, A. Voultsios, M. D. Salkeld, D. J. Kennaway

Research Centre for Reproductive Health, University of Adelaide, Adelaide, SA, Australia

In Australia, 17% of the working population, or 1.4 million Australians, are working some form of shift work (1). There is strong evidence implicating shift work in the development of many chronic diseases including coronary heart disease, obesity, diabetes and other metabolic

disturbances (2-4). Additionally, shift work during pregnancy is associated with poor pregnancy outcomes including preterm birth, low birth weight and spontaneous abortion (5-9). Consequently, we conducted a comprehensive examination of the effect of a simulated shift work schedule during prenatal development on the health of the offspring in an animal model. Pregnant rats were exposed to a simulated shift work schedule throughout gestation and the first week after birth. The offspring were assessed for a range of health parameters including weight, growth, circadian rhythm function, neurobehavioural development and metabolic health of the offspring as adults. We found that while there was no effect on birth weight, the metabolic health of the offspring was significantly altered. Male pups born to dams subjected to the shift work schedule had increased adiposity and increased plasma leptin concentration at 4 months of age. Interestingly, plasma leptin concentrations were elevated in the shift work group above that of the controls during time of feeding (at night). In a subset of rats allowed to continue to 12 months of age glucose tolerance and insulin sensitivity was significantly impaired in the female offspring. These results suggest that exposure to shift work during pregnancy may have significant consequences for the offspring as adults.

(1) Statistics, A.B.o., Working Time Arrangements. 2006. 6342.
(2) Kawachi, I., et al., 1995. 92(11): p. 3178-3182.
(3) Suwazono, Y., et al.,. Journal of Occupational & Environmental Medicine, 2006. 48(5): p. 455-461.
(4) De Bacquer, D., et al.,. Int J Epidemiol, 2009. 38(3): p. 848-54.
(5) McDonald, A.D., et al., 1988. 45(1): p. 56-62.
(6) Zhu, J.L., N.H. Hjollund, and J. Olsen,. Am J Obstet Gynecol, 2004. 191(1): p. 285-91.
(7) Lawson, C.C., et al., Am J Obstet Gynecol, 2009. 200(1): p. 51 e1-8.
(8) Zhu, J.L., et al., Journal of Occupational & Environmental Medicine, 2004. 46(11): p. 1144-9.
(9) Whelan, E.A., et al., Epidemiology, 2007. 18(3): p. 350-5.

240

CALCIUM SUPPLEMENTATION DOES NOT RESCUE THE PROGRAMMED ADULT BONE DEFICITS ASSOCIATED WITH PERINATAL GROWTH RESTRICTION

M. E. Wlodek[1], J. D. Wark[2], T. Romano[1,2]

[1]Physiology, The University of Melbourne, Parkville, VIC, Australia
[2]Medicine, The University of Melbourne, Bone and Mineral Service, Royal Melbourne Hospital, Parkville, VIC, Australia

Low birth weight programs adult diseases. We have previously reported that offspring born small, resulting from uteroplacental insufficiency, have shorter femurs, lower bone mineral content and bone strength as adults. We determined the effects of calcium supplementation from adolescence on growth restricted male and female offspring.

Bilateral uterine vessel ligation (Restricted) or sham surgery (Control) was performed on gestational day 18 (term=22 days) in rats inducing uteroplacental insufficiency and growth restriction. At 2 months pups were allocated to diet groups: 1-constant normal calcium (0.46%), 2-variable normal calcium (0.46%), 3-constant high calcium (2.4%), 4-variable high calcium (2.4%). Diet groups 1 and 3 consumed their diets constantly. Groups 2 and 4, rats consumed one diet for 5 days, switching to a low calcium diet for the next 5 days. At post-mortem (6 months), dual energy xray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT ; for true volumetric trabecular and cortical mineral content, density, dimensions and stress strain in dex) were performed on the femur.

Male and female Restricted offspring were born 14% lighter than Controls; females remained smaller at 6 months ($p<0.05$). Restricted males and females had reduced trabecular and cortical bone mineral content , regardless of diet ($p<0.05$). Trabecular bone mineral density was lower in Restricted females ($p<0.05$). Consuming constant high calcium increased cortical bone mineral content in Restricted males and both female groups ($p<0.05$). Stress strain index of bone strength was lower in Restricted offspring, regardless of diet. DXA results matched pQCT.

Being born small programs reduced adult femur length, dimensions and strength. High constant calcium increases adult cortical bone mineral density in low birth-weight offspring and normal-weight females but did not rescue the programmed bone deficit.

241

PLACENTAL RESTRICTION ALTERS MICRORNAS EXPRESSION IN SKELETAL MUSCLE, LIVER AND ADIPOSE TISSUE YOUNG AND ADULT OFFSPRING IN THE SHEEP

H. Harryanto, M. J. De Blasio, P. A. Grant, K. L. Gatford, J. S. Robinson, J. A. Owens

Discipline of Obstetrics & Gynaecology, Research Centre for Early Origins of Health & Disease, Robinson Institute, Unive, Adelaide, SA, Australia

Introduction: Placental restriction (PR) is a major cause of intrauterine growth restriction (IUGR), which is associated with adult onset type 2 diabetes. This appears mediated through down-regulation of gene and/or protein expression of insulin signalling pathway in insulin sensitive tissues. MicroRNAs (miRNAs) are small non-protein coding RNA that can down-regulate expression of multiple target mRNAs and/or proteins. We hypothesised that PR alters of miRNAs expression in insulin sensitive tissues of progeny, some of which target the insulin signalling pathway, in young (44 days) and adult (18 months) progeny.

Methods: Placental growth in sheep was restricted by removal of most uterine implantation sites from non-pregnant Merino ewes, to reduce subsequent placental size and function (1). Total RNA was extracted from vastus lateralis, liver and perirenal fat and miRNA expression analysed by microRNA Exiqon array, then verified by qRT-PCR. Predicted targets were identified by miRecords database then subjected to Ingenuity Pathway Analysis (IPA) to identify any targeted networks.

Results: In young offspring, PR reduced expression of some miRNAs in liver and vastus lateralis, but increased some in perirenal fat. In adult offspring, PR usually increased miRNAs expression in liver, vastus lateralis and perirenal fat. Some differentially expressed miRNAs were predicted to target insulin signalling molecules, such as INSR (miR-451 and -597), IRS1 (miR-487b) and p85α (miR-21 and -324-3p); others to target regulatory and ancillary molecules, such as PPARα (miR-17-5p, -21 and -144), IGF1 (miR-1) and FOXO1 (miR-142-3p and -144). Differential expression of some miRNAs (miR-1 and -17-5p) could potentially involve altered DNA methylation as indicated by the presence of CpG islands.

Conclusion: PR does alter expression of miRNAs in insulin sensitive tissues of offspring, but variably with age. Differential expression of miRNAs in insulin sensitive tissues of PR offspring may contribute to their altered insulin sensitivity, which also changes with age (2-3).

(1) Robinson JS et al (1979) J Dev Physiol 1(5): 379-398
(2) De Blasio MJ et al (2007) Endocrinology 148(3): 1350-1358
(3) Owen JA et al (2007) AM J Physiol 292(6): E1879-E1889

242

MATERNAL FOLIC ACID SUPPLEMENTATION AND ABUNDANCE AND EXPRESSION OF INSULIN-LIKE GROWTH FACTOR-II IN OFFSPRING

E. Mohamed Jamil, P. A. Grant, S. Moretta, W. C. Kong, K. L. Gatford, J. A. Owens

Robinson Institute, School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, SA, Australia

Background: Folic acid supplementation (FAS) is recommended for pregnant women in order to prevent neural tube defects in babies. By supplying methyl groups, folic acid may also affect methylation of DNA and histones thus affecting gene expression. We have recently shown that increasing methyl group supply through maternal FAS (MFAS) alters IGF-II abundance in offspring, increasing plasma IGF-II, before weaning and mainly in females.

Aims/Hypothesis: The aims were to characterise the effect of MFAS on *igf2* expression in tissues and the associations with plasma IGF-II in young offspring.

Methods: Female Wistar rats were fed either a control (2mg.kg^{-1}) (n=22) or a folic acid supplemented diet (6mg.kg^{-1}) (n=23) from two weeks prior to mating until offspring were born. Offspring were sacrificed at postnatal day 7, and offspring plasma and skeletal muscle, liver, lung, kidney, heart and three fat depots (omental, retroperitoneal and subcutaneous) collected. Plasma IGF-II was measured by specific radioimmunoassay (RIA) after molecular sieving by high performance liquid chromatography (HPLC). Quantitative RT-PCR was used to measure *igf2* gene expression, normalized to the expression of β-actin.

Results: MFAS increased plasma IGF-II in offspring overall (x1.9, p=0.004), mainly in females (x3.4), at day 7 (MFAS x Sex, p=0.02). MFAS tended to increase skeletal muscle *igf2* expression in females (x2.8), but not males, at day 7 (MFAS x Sex, p=0.06). In males, plasma IGF-II was positively associated with igf2 expression in liver and lung and negatively with that in kidney and omental fat (MLR, p=0.02, r^2=0.70), while in females, plasma IGF-II was positively associated with igf2 expression in skeletal muscle, liver and heart and negatively associated with that in lung (MLR, p=0.15, r^2=0.570).

Conclusions: MFAS increases IGF-II abundance postnatally in offspring, but in a sex dimorphic manner and possibly through actions on skeletal muscle.

243

BEING BORN SMALL PROGRAMS NEPHRON DEFICITS AND HYPERTENSION IN THE NEXT GENERATION

M. E. Wlodek[1], L. A. Gallo[1], M. Q. Mazzuca[1], L. A. Cullen-McEwen[3], K. M. Moritz[2]

[1]Physiology, The University of Melbourne, Parkville, VIC, Australia
[2]School of Biomedical Sciences, University of Queensland, Brisbane, QLD, Australia
[3]Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia

Intrauterine growth restriction caused by uteroplacental insufficiency increases the risk of cardiovascular and kidney disease in adulthood. We have reported nephron deficits in growth restricted male and female rats at 6 months, with glomerular hypertrophy and hypertension only in males. Our aim was to explore whether nephron deficits and hypertension associated with F1 growth restriction are evident in the next (F2) generation.

Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted, R) or sham surgery (Control, C) on day 18 of pregnancy in WKY rats (F0). F1R and F1C females (F1) were mated with normal males. In F1 pregnant mothers, blood pressure (tail cuff) and 24-hr renal function (plasma and urine electrolytes) were measured on gestational day 18 and 19-20, respectively. F2 offspring weight and nephron number (unbiased stereology) were measured at 20 days gestation and F2 blood pressure (tail cuff) measured at 6 months.

F1 female blood pressure and renal function during pregnancy were not different between groups. F2 fetal weight was not different in males, but F2R females were smaller than controls (p<0.05) with no differences in placental weight. F2R male fetuses had 20% fewer nephrons (606±35) compared to F2C (764±44) (n=3 from different litters; p<0.05). F2 female nephron number is under analysis. At 6 months, F2R males had increased blood pressure (153±4mmHg) compared to controls (133±5mmHg) (n=10 from different litters; p<0.05), while female blood pressure was not different.

N ephron deficits and hypertension associated with uteroplacental insufficiency occur in the next generation of male offspring, in the absence of reduced fetal weight. Alterations in maternal blood pressure or renal function during pregnancy were not associated with this intergenerational programming.

244

PERICONCEPTIONAL UNDERNUTRITION ALTERS FETAL PANCREATIC B-CELL MASS IN LATE GESTATION

H. Liu, F. H. Bloomfield, M. H. Oliver, K. L. Connor, D. M. Sloboda

Liggins Institute and the National Research Centre for Growth and Development, University of Auckland, Auckland, New Zealand

Early life adversity, such as maternal undernutrition, has been shown to have long-term consequences, resulting in increased risk of obesity and metabolic disease in offspring(1). It is now clear that the critical developmental window of vulnerability includes the periconceptional period and undernutrition at this time influences fetal metabolic status in late gestation(2, 3). In the sheep, early pregnancy undernutrition altered pancreatic responses to glucose and amino acids, suggesting advanced β-cell maturation(4). We therefore investigated the effects of periconceptional maternal undernutrition (PCUN) on β-cell development in the late gestation fetus. METHODS: Pancreata were collected from 121d old singleton fetal sheep (term, 147d) representing a critical period of pancreatic maturation. Mothers were either well fed (ad libitum) or undernourished (reduced feed to maintain 10-15% reduction in ewe bodyweight) around the time of conception (60d before mating until 30d after). Pancreatic mRNA levels of pro-insulin, pro-glucagon, GLUT-2, IGF-2, and activated caspase-3 were measured using qPCR. Measures of islet area, β-cell mass and apoptosis were performed after immunohistochemical localisation of insulin, glucagon, and activated caspase-3 proteins. RESULTS: In female fetuses, PCUN resulted in an increased presence of apoptotic cells in pancreatic islets but was not associated with significant changes in β-cell mass. Interestingly, compared to controls, undernourished female fetuses demonstrated increased small:large islet ratio suggesting an increased proportion of smaller, mature islets. In contrast, fetal β-cell mass was significantly reduced by PCUN in male fetuses and was associated with decreased small:large islet ratio suggesting a maintenance of large immature islets. Expression of pro-insulin, pro-glucagon, GLUT-2, IGF-2, and caspase-3 were similar between groups. CONCLUSIONS: PCUN has sexually dimorphic effects on pancreatic islets in the late gestation ovine fetus. Accelerated maturation of β-cells or reduced β-cell mass in response to PCUN could contribute to loss of β-cell reserve in later life, predisposing the individual to metabolic risk.

(1) Plagemann, A., J Matern Fetal Neonatal Med, 21(3): p. 143-8 (2008)
(2) Bloomfield, F.H., et al., Endocrinology, 145(9): p. 4278-85 (2004)
(3) Connor, K.L., et al., Reprod Sci. Jun;16(6):573-83 (2009)
(4) Oliver, M.H., et al., Pediatr Res. Apr;57(4):591-8 (2005)

245

NEONATAL EXENDIN-4 TREATMENT INCREASES BETA-CELL MASS AND ALTERS ISLET GENE EXPRESSION IN THE IUGR LAMB.

S. A. Sulaiman[1], K. L. Gatford[1], S. N.B. Mohammad[1], M. J. De Blasio[1], M. L. Harland[1], R. A. Simmons[2], J. A. Owens[1]

[1]Robinson Institute and School of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, SA, Australia
[2]Department of Paediatrics and School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background: IUGR increases the risk of Type 2 diabetes (T2DM) in later life, due to impaired insulin sensitivity and limited adaptation to this of insulin secretion. In placentally-restricted IUGR rats, these adverse sequelea including development of T2DM can be prevented by neonatal administration of exendin-4. We have also found that in naturally fetal-growth-restricted twin lambs, exendin-4 treatment increases insulin secretion.

Aim/Hypothesis: To determine if exendin-4 treatment of the twin IUGR lamb increases insulin secretion by increasing beta-cell mass, function or both and the molecular basis of any changes.

Methods: Twin IUGR lambs were injected s.c. daily with vehicle (n=8) or exendin-4 (1 nmol.kg-1, n=8), and control singleton lambs were injected with vehicle (n=7), from 1 to 16 d of age. At day 16, pancreas was collected and infused with glucose solution and collagenase for digestion and isolation of the islet, at 35 ºC for 40 minutes in water bath. Islets were stored at -80 ºC for analysis of gene expression of selected islet regulatory and functional genes using Real Time PCR, normalised to beta-actin expression.

Results: Twinning IUGR did not alter beta-cell mass in lambs, but increased islet expression of glucokinase ($p=0.019$) and tended to increase glut2 ($p=0.08$), while exendin-4 treatment increased beta-cell mass, and igf1R expression ($p=0.08$). Islet expression of genes directly and indirectly regulated by pdx-1, glut2, glucokinase, K+ channel subunit kir6.2, Ca2+ Channel subunit CACNa1D, also correlated positively with pdx-1 ($r=0.621$, $p=0.002$; $r=0.435$, $p=0.028$; $r=0.693$, $p=<0.001$; $r=0.396$, $p=0.042$, respectively).

Conclusions: Increased islet expression of glucokinase and glut2 in twin IUGR lambs may indicate compensation to increase insulin secretory capacity and exendin-4 treatment increases beta-cell mass in twin IUGR lambs, possibly in part by increased IGF responsiveness.

246

BEING BORN SMALL PROGRAMS FETAL GROWTH RESTRICTION AND PANCREATIC DEFICITS IN THE SUBSEQUENT GENERATION

M. Tran[1], K. M. Moritz[2], L. A. Gallo[1], K. T. Westcott[1], A. Jefferies[1], M. E. Wlodek[1]

[1]Department of Physiology, The University of Melbourne, Parkville, VIC, Australia
[2]School of Biomedical Sciences, University of Queensland, St Lucia, QLD, Australia

Intrauterine growth restriction increases risk of adult metabolic disease. We have shown that growth restriction alters insulin sensitivity but not glucose tolerance in 6 month old females. The normal metabolic adaptations to pregnancy may be altered in females born small. The aim of this study was to determine whether growth restricted females develop metabolic dysfunction during pregnancy and whether this has pancreatic implications in the next generation. Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted) or sham surgery (Control) on day 18 of pregnancy in WKY rats (F0). Control and Restricted non pregnant females were studied at 4 months of age. Another cohort of Control and Restricted females were mated and studied during pregnancy. Non pregnant and pregnant (day 18) females underwent an intraperitoneal glucose tolerance test (IPGTT, 1g/kg). Pancreatic tissue was sectioned and immunostained for insulin to determine β-cell mass. F2 fetal weight and pancreatic β-cell and islet volume density was measured at day 20 of pregnancy. F1 Restricted non pregnant females had lower plasma insulin levels in the fed state ($p<0.05$) which was associated with reduced β-cell mass (-37%; $p<0.05$). Restricted females during pregnancy had impaired glucose tolerance but β-cell mass was not different to Controls. F2 Restricted female fetuses at day 20 had lower body weight ($p<0.05$), with Restricted males having reduced β-cell and islet volume density (n=3/group from different litters). Non pregnant Restricted females had lower plasma insulin levels and decreased β-cell mass. During pregnancy, Restricted females had impaired glucose tolerance but normal pancreatic β-cell function suggesting a compensatory mechanism that masks this deficit. Fetal growth restriction and pancreatic deficits, associated with uteroplacental insufficiency, occurs in the subsequent generation of male offspring. Thus being born small alters the metabolic profile during pregnancy and influences growth and pancreatic development of the next generation.

247

AGE-RELATED CHANGES IN THE REGULATION OF SLEEP AND THE MALE GONADAL AXIS

P. Y. Liu

Woolcock Institute of Medical Research, University of Sydney and Concord Hospital, NSW, Australia

Endocrine glands communicate continuously as well as through intermittent signal exchange. Pulsatile (intermittent) signals allow rapid large adjustments to maintain homeostasis in the presence of potentially catastrophic environmental and other changes. Pulses of growth hormone and luteinising hormone in particular orchestrate growth, development and reproduction, whereas melatonin and cortisol coordinate circadian rhythm. Hormonal disruption, through altered pulsatile secretion or abnormal network regulation, can therefore lead to disease. Quantifying pulsatile secretion and assessing network hormonal function requires: (1) deconvolution methods that are both empirically and mathematically validated; (2) experimental interventions that "clamp" hormonal signals through a physiologically relevant range of dose-responses; and (3) analyses and mathematical models that capture linear, non-linear and entropic relationships. Such investigations in the testicular axis unveil impaired gonadotrophin-releasing hormone action, attenuated luteinising hormone drive of testosterone secretion, opposing effects of testosterone feedback on luteinizing hormone secretion and network dysynchrony as key features of male reproductive aging.

Sleep duration, architecture and quality are also age-dependent whereas the secretion of many hormones, including growth hormone and luteinising hormone is sleep-dependent. Fundamental relationships among all three of sleep, ageing and gonadal axis regulation must therefore exist but elucidation requires further systematic investigations. Quantifying sleep architecture through power spectral analysis and combining this with mathematical deconvolution of hormonal secretion will allow novel insights into the endocrinology of sleep and its changes with aging. These modeling approaches will require a collaborative and interdisciplinary team of researchers studying frequently and intensively assessed cohorts of normal and diseased individuals. Initial studies in men with obstructive sleep apnoea are currently underway and studies in normal individuals in whom sleep or sleep architecture is manipulated are planned.

248

CLINICAL PHARMACOLOGY OF HUMAN CHORIONIC GONADOTROPHIN: RANDOMIZED SEQUENCE CROSS-OVER STUDY OF RECOMBINANT *VS* URINARY HCG IN GONADOTROPHIN SUPPRESSED HEALTHY MEN

A. Idan[1], T. Sivanathan[2], A. J. Conway[1,2], L. Turner[2], D. J. Handelsman[1,2]

[1]ANZAC Research Institute, University of Sydney, Sydney, NSW, Australia
[2]Andrology, Concord Hospital, Sydney, NSW, Australia

Human chorionic gonadotrophin (hCG) has an essential therapeutic role in gonadotrophin deficient men to maintain physiological serum and testicular testosterone (T) levels required to induce somatic androgenisation, spermatogenesis and male fertility. For over 6 decades, urinary hCG (uhCG) has been purified from 1st trimester pregnancy urine but, of 9 commercial brands available in 1947, only one remains marketed in Australia. Regulatory agencies encourage replacement of human-origin biological products with recombinant alternatives to minimize risk of transmissible human disease. However, recombinant hCG (rhCG) is marketed only for triggering ovulation so

comparisons with uhCG are reported only for ovulation induction. If uhCG were unavailable, treatment for gonadotrophin deficient infertile men would require rhCG but virtually no clinical pharmacology data on rhCG are available in men. Therefore, we compared the pharmacokinetics and pharmacodynamics of rhCG and uhCG directly in a investigator-initiated cross-over clinical study without commercial sponsor. Eight healthy eugonadal men (age 34±4 yr, height 176±2 cm, weight 80.5±2.7 kg, BMI 25.94±0.9 kg/m^2, BSA 1.98±0.04 m^2, baseline serum T 20.4±3.6 nmol/L, LH 3.4±0.6 IU/L, FSH 3.9±0.9 IU/L) with nandrolone-suppressed endogenous gonadotrophin and testosterone secretion were administered a single subcutaneous dose of uhCG (1500 IU) and rhCG(62.5 µg, ~1500 IU) a week apart in random sequence with blood sampling before and at 24, 48, 72, 96 and 168 hours after each injection. There were no significant differences in time course of serum hCG (immunoflurometric assay) or for T, DHT or E2 (LC tandem MS assay) by repeated measures ANOVA nor in time of peak, peak concentration or area under curve for serum hCG (median, IQR; 24 hr, 24-24hr; 31 IU/L, 24-39 IU/L; 2244 IU*hr/L, 1502-2620 IU*hr/L, respectively) or T (72 hr, 72-72 hr; 23 nmol/L, 19-36 nmol/L; 2700 nmol*hr/L, 2005-4143 nmol*hr/L). We conclude that the two hCG products have clinically equivalent single dose pharmacokinetics and pharmacodynamics so they can be used interchangeably on a dose equivalent basis.

249

PROOF THAT HUMAN AR Q-TRACT LENGTH DETERMINES ANDROGEN SENSITIVITY IN VIVO

U. Simanainen[1], D. Das[2], M. Brogley[2], Y. Gao[1], M. Jimenez[1], T. D. Harwood[1], D. J. Handelsman[1], D. M. Robins[2]

[1]Andrology, ANZAC Research Institute, Concord, Australia

[2]Department of Human Genetics, University of Michigan Medical School, Ann Arbor, United States

Androgens via the androgen receptor (AR) mediate the regulation of hypothalamic-pituitary-testicular axis by negative feedback of serum gonadotrophin and testosterone levels. The variable length CAG triplet repeat polymorphism encoding a polyglutamine stretch (Q-tract) in exon 1 of the human AR is thought to determine human AR (hAR) androgen sensitivity, based on an inverse relationship with *in vitro* AR transcriptional activity as supported by some but not all epidemiological studies, yet direct *in vivo* proof is lacking. To overcome the limitations of artificial *in vitro* experiments and observational studies, we used knock-in mouse lines containing hAR alleles ("humanized" AR) with short (12Q), median (21Q) or long (48Q) Q-tract variations (1). In mature male mice 14 days after orchidectomy with subdermal depot DHT implants for the last 5 days, suppression of castrate serum LH levels demonstrated a significant rank order of suppression according to Q tract length [12Q (94%) > 21Q (74%) > 48Q (52%) ($p<0.005$ test for trend)] despite similar levels of serum DHT and its 3aDiol and 3βDiol metabolites (by LC tandem MS). Similar significant rank order trends according to Q-tract length were evident for weights of all prostate lobes (anterior, dorsolateral, ventral) as well as seminal vesicles (SV) (full, emptied) in orchidectomized mice with or without DHT treatment. These findings demonstrate significant effects of AR Q-tract length mediating DHT responsiveness of post-castration serum LH levels and androgen-dependent organ weights. These experiments in this paradigm of castrate DHT-replaced mice provide the first direct proof that hAR Q-tract length inversely determines *in vivo* androgen sensitivity, and suggest a mechanism by which AR Q-tract length differences impact multiple aspects of human health and disease.

(1) MA, Scheller A, Brogley M, Robins DM (2006) Mol Endocrinol. 20(6):1248-60.

250

ANDROGEN DEPRIVATION THERAPY FOR PROSTATE CANCER INCREASES VISCERAL FAT MASS AND INSULIN RESISTANCE

E. J. Hamilton[1,2], E. J. Gianatti[1,2], B. Strauss[3], J. Wentworth[4], D. Lim Joon[5], D. Bolton[6], J. D. Zajac[1,2], M. Grossmann[1,2]

[1]University of Melbourne Austin Health, Heidelberg, VIC, Australia

[2]Endocrine Unit, Austin Health, Heidelberg, VIC, Australia

[3]Monash University, Clayton, VIC, Australia

[4]Walter and Eliza Hall Institute, Parkville, VIC, Australia

[5]Radiation Oncology Austin Health, Heidelberg, VIC, Australia

[6]Urology Austin Health, Heidelberg, VIC, Australia

Introduction: Androgen deprivation therapy (ADT) for treatment of prostate cancer increases fat mass and may increase risk of developing type 2 diabetes, but the relationship between sex steroid deficiency and abdominal fat distribution is controversial. Aim: We hypothesized that ADT increases visceral abdominal fat and insulin resistance. Methods: 26 males, mean age 70.6 (± 6.8) years, with non-metastatic prostate cancer were studied at baseline, 6 and 12 months after commencing ADT. Abdominal fat distribution was assessed using Slice-O-Matic software analysis of L4-5 computed tomography images. Results: ADT decreased total testosterone (12.8 ± 0.8 to 0.7 ± 0.1 nmol/L), and estradiol (103.7 ± 8.0 to 30.1 ± 3.5 pmol/L; all $p<0.001$). After 12 months ADT, mean lean body mass decreased by 1.9 kg, and mean fat mass increased by 3.4 kg (all $p<0.001$). Subcutaneous fat area (240.7 ± 107.5 to 271.27 ± 92.8cm2, $p=0.001$) and visceral fat area (160.8 ± 61.7 to 195.9 ± 69.7cm2, $p=0.001$) increased, as did total cholesterol (4.7 ± 1.0 to 5.3 ± 1.1 mmol/L, $p=0.002$), triglycerides (1.1 ± 1.5 to 1.6 ± 1.1 mmol/L $p=0.01$), and HOMA-IR (2.50 ± 1.1 vs. 2.79 ± 1.3, $p<0.05$). Fasting glucose level and HbA1c did not change. Twelve months of ADT increased Leptin (12.5 ± 13.8 to 18.8 ± 15.5ng/mL, $p=0.007$) whereas Adiponectin increased at 6 months (21.6 ± 12.4 to 30.2 ± 22.7mg/mL, $p=0.025$), but not at 12 months. Conclusions: ADT-induced sex steroid deficiency is associated with accumulation of visceral fat and insulin resistance. Understanding the physiological changes associated with ADT should 1) aid identification of those at highest risk of diabetes and cardiovascular events, and 2) inform about the risk-benefit ratio of ADT for the treatment of prostate cancer.

251

LONGER-TERM EFFECTS OF TESTOSTERONE THERAPY ON SLEEP, BREATHING AND BODY COMPOSITION IN OBESE MEN WITH OBSTRUCTIVE SLEEP APNEA (OSA) UNDERGOING WEIGHT LOSS: A RANDOMISED PLACEBO CONTROLLED 18 WEEK TRIAL

C. Hoyos[1,2], B. Yee[1,2], C. Phillips[1], R. Gruntein[1,2], P. Liu[1,2]

[1]Endocrine and Cardiometabolic Group, Woolcock Institute of Medical Research, Sydney, NSW, Australia
[2]Central Clinical School, University of Sydney, Sydney, NSW, Australia

Introduction: Testosterone (T) therapy reduces fat and increases muscle. Such body compositional changes in men with OSA should improve sleep disordered breathing. However we previously showed that high dose T therapy acutely worsens breathing during sleep. The effects of longer-term, more physiological T therapy on weight, body composition, sleep and breathing are unknown.

Methods: Obese men with OSA were randomised (n=67), in a 18-week double-blind placebo controlled parallel group study, to 3 IM injections (0, 6, 12 weeks) of either 1000mg Testosterone undecanoate (n=33, BMI=36.6±0.8kg/h^2, apnea hypopnea index (AHI) =33.2±3.9 events/h) or placebo (n=34, BMI=34.9±0.7kg/h^2, AHI=30.3±2.7 events/h). Anthropometry, body composition (abdominal CT and whole body DEXA scans), overnight polysomnography and arterial stiffness were measured before, during and after the treatment period. Data were analysed by mixed models adjusted for baseline weight and are mean (or mean of change from baseline)±SEM.

Results: T treatment (vs placebo) increased blood T (by 7.0nM overall, p<0.0001) and suppressed gonadotropins (by 3-4IU/L, p<0.0001 for each), as expected. Body composition improved overall with diet and exercise (each p<0.05), but was similar between groups: weight (T= -1.8±0.3kg, placebo=- 2.1±0.3 kg); visceral abdominal fat (T=-29.6±18.9cm^3, placebo=-47.1±11.6cm^3) and; total body fat (T=-3114±413g, placebo=-2861±566g). Lean muscle mass significantly increased in the T (1194±293g) compared to the placebo (-408±320g, p=0.001) group. T treatment worsened breathing during sleep acutely: oxygen desaturation index (3%) (p=0.02) and saturation time <90%) (p=0.03), total AHI (p=0.03) and non-REM AHI (p=0.045), but not after 18 weeks. However, markers of cardiometabolic risk such as arterial stiffness (p=0.035) and liver fat (p=0.046) both consistently improved with T therapy, as did sexual desire (p=0.01).

Conclusion: T therapy increases lean mass, improves arterial stiffness and reduces liver fat. Sleep disordered breathing increased acutely but not in the longer term. T may improve cardiometabolic risk in the longer term despite acutely worsening sleep apnea

252

LOW URINARY IODINE POSTPARTUM IS ASSOCIATED WITH HYPOTHYROID POSTPARTUM THYROID DYSFUNCTION AND PREDICTS LONGTERM HYPOTHYROIDISM

B. G.A. Stuckey[1,2,3], G. N. Kent[4], J. R. Allen[4], L. C. Ward[2], S. J. Brown[2], J. P. Walsh[1,2,3]

[1]Keogh Institute for Medical Research, Nedlands, WA, Australia
[2]Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia
[3]School of Medicine and Pharmacology, University of Western Australia, Nedlands, WA, Australia
[4]Clinical Biochemistry, PathWest Laboratory Medicine, Nedlands, WA, Australia

Background: Postpartum thyroid dysfunction (PPTD) is a lymphocytic thyroiditis characterised by a short hyperthyroid phase followed, with a peak prevalence at 6 months, by a hypothyroid phase which carries a risk of longterm hypothyroidism. Iodine availability has a major effect on thyroid function, however Western Australia has been shown to be an iodine-replete state.

Aims: We aimed to examine the relationship between iodine status and firstly PPTD and secondly longterm hypothyroidism.

Methods: In 1995 we conducted a survey of 748 women at 6 months postpartum. We identified 86 women with PPTD. Each was matched with a euthyroid control and invited to attend for clinical assessment. 149 women of this case-control cohort (74 PPTD and 75 controls) provided a sample for urinary iodine concentration (UIC). In 2007, 98 of those women attended for the follow-up study.

Results: At 6 months postpartum, the median UIC (quartiles) μg/L, for observed TSH ranges were significantly different:- for TSH<0.4mU/L 130.0 (82.0,170.0); for TSH 0.4-4.0mU/L 123.0 (80.5,168.0); for TSH >4.0 mU/L 85.0 (40.0,141.5), p=0.018. The odds of hypothyroid PPTD were associated with decreasing log iodine (OR 2.54, 95%CI: 1.47, 4.35), and a UIC <50 μg/L (OR 4.22, 95%CI: 1.54, 11.55). In the longterm analysis, logUIC significantly predicted hypothyroidism at 12 year follow-up (p=0.002); as did UIC <100μg/L (p = 0.03) and UIC < 50 μg/L (p = 0.02). The association was independent of antibody status or breastfeeding.

Conclusion: Given the established iodine-sufficient status of our geographical area we suggest that low UIC measured at 6 months postpartum is not indicative of a dietary influence on either PPTD or longterm hypothyroidism in our study population. Rather, we believe that it is a marker for greater destructive thyroiditis with discharge of thyroidal iodine in the preceding hyperthyroid phase and is, thereby, predictive of a greater risk of longterm hypothyroidism.

253

MANDATORY IODINE FORTIFICATION OF BREAD IN AUSTRALIA: IODINE STATUS OF PREGNANT WOMEN

P. A. Fogarty, B. S. Hetzel, T. J. Nettelbeck, R. Roberts, N. A. Hodyl, D. J. Torpy, J. Couper, V. L. Clifton

Pregnancy and Development Group, Robinson Institute, Lyell McEwin Hospital, Univ, ELIZABETH VALE, SA, Australia

The Australian population is mildly iodine deficient which can impact on fetal growth and development during pregnancy. The World Health Organization recommendation for adequate iodine intake during pregnancy based on median urinary iodine concentrations (UIC) is 150-249µg/L. In an attempt to increase iodine intake in the Australian population, bread has been mandatorily fortified with iodine since October 2009. We were interested in assessing the effect of iodine fortification of bread and iodine supplements on the iodine status of pregnant women. Pregnant women (n=103) were recruited prospectively at the antenatal clinic of the Lyell McEwin Hospital in Adelaide before and after commencement of iodine fortification in October 2009. Urine samples were collected at 12, 18, 30 and 36 weeks' gestation and assayed for iodine levels. Multivitamin supplement use during pregnancy was noted. Median UIC prior to iodine fortification of bread was 75 µg/L. Median UIC following fortification was significantly higher than pre-intervention (116 µg/L, $p<.001$). Median UIC of women taking iodine supplements was significantly higher than in women not taking iodine supplements (142 µg/L vs. 80 µg/L, $p<.001$). Pre-intervention median UIC of women taking iodine supplements was significantly higher than in women not taking iodine supplements (96.5 µg/L vs. 68 µg/L, $p=.018$). The iodine status of women taking iodine supplements significantly improved following fortification of bread from 96.5 µg/L to 163.5 µg/L ($p=.008$), indicating an adequate iodine intake. The iodine status of women not taking iodine supplements also improved from 68µg/L to 84µg/L ($p=.011$), but they remained iodine-deficient. There was a small but significant improvement in iodine status associated with mandatory iodine fortification of bread. However, most women remain iodine deficient unless taking a multivitamin supplement that includes iodine. Pregnant women, with the exception of those with pre-existing thyroid disease, should consider taking an iodine supplement.

254

IS THE PHASE OF THE MENSTRUAL CYCLE IMPORTANT WHEN SCREENING FOR PRIMARY ALDOSTERONISM IN WOMEN, AND DOES RENIN ASSAY METHOD MATTER?

A. H. Ahmed[1], R. D. Gordon[1], P. J. Taylor[1], G. Ward[2], E. Pimenta[1], M. Stowasser[1]

[1]Endocrine Hypertension Research Centre, Princess Alexandra Hospital, Woolloongabba, QLD, Australia

[2]Endocrinology, Sullivan & Nicolaides Pathology, QLD, Australia

Background: The most popular screening test for primary aldosteronism (PAL) is the plasma aldosterone/renin ratio (ARR). Because fluctuations in both estrogen and progesterone affect aldosterone and renin levels, we studied effects of phase of the menstrual cycle on ARR, measuring renin as both direct renin concentration (DRC) and plasma renin activity (PRA), and comparing with male levels.

Methodology: Normotensive, non-medicated female volunteers (n=19) had blood and urine collected midmorning for measurement of plasma aldosterone (by mass spectrometry), DRC, PRA, serum estrogen, progesterone, and urinary aldosterone during menses and 10±1 (SD) and 20±1 days after the first day of the last menstrual period. Normal males (21) also studied.

Results: Compared with follicular phase (day 10), luteal (day 20) concentrations were higher for serum progesterone [median 0.5 (range 0.3-7.6) vs 39.8 (12.1-71.5) nmol/L, P<0.001], plasma aldosterone [170 (133-524) vs 454 (181-1141) pmol/L, P<0.001], urinary aldosterone [105 (26-334) vs 233 (16-551) pmol/mmol creatinine, P<0.01], DRC [28 (10-58) vs 38 (15-78) mU/L, P<0.01], PRA [2.1 (1.0-5.7) vs 3.8 (1.6-9.2) ng/ml/hr, P<0.001] and ARR calculated using DRC [8.3 (2.3-49.9) vs 14.2 (2.3-75.7), P<0.001] but not using PRA [109 (24-227) vs 133 (30-300), P>0.05]. In two subjects the luteal ARR (using DRC) rose above the cutoff for normal (70) used in our laboratory. ARR (using DRC or PRA) was slightly (but not significantly) lower during menses than at day 10. Females had significantly higher ARR than healthy males regardless of menstrual phase. The difference was smallest during menses.

Conclusions: Luteal levels of renin and aldosterone are highest, due to progesterone antagonizing aldosterone action. Greater increase in PRA than DRC results in ARR (PRA) not increasing, while ARR (DRC) does increase, with risk of false positives.

255

ASSESSMENT OF THE HYPERGLYCAEMIC EFFECT OF PREDNISOLONE DURING AN EXACERBATION OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE WITH A CONTINUOUS GLUCOSE MONITORING SYSTEM

M. G. Burt[1,4], G. W. Roberts[2,4], N. R. Aguilar-Loza[1], P. Frith[3,4], S. N. Stranks[1,4]

[1]Southern Adelaide Diabetes and Endocrine Services, Repatriation General Hospital, Adelaide, SA, Australia

[2]Pharmacy Department, Repatriation General Hospital, Adelaide, SA, Australia

[3]Department of Respiratory Medicine, Repatriation General Hospital, Adelaide, SA, Australia

[4]Faculty of Health Science, Flinders University, Adelaide, SA, Australia

Glucocorticoids are frequently prescribed to treat exacerbations of inflammatory disease, including chronic obstructive pulmonary disease (COPD). While Cushing's syndrome predominantly causes postprandial hyperglycaemia[1], the pattern of hyperglycaemia induced by

exogenous glucocorticoids in hospitalized patients has not been well described. The aim was to produce a detailed assessment of the effect of prednisolone on glucose concentration to optimize management of prednisolone-induced hyperglycemia.

Forty consecutive consenting patients without known diabetes admitted to hospital with an exacerbation of COPD and treated acutely with prednisolone (Group 1, 25 men, age = 77±14 years, prednisolone = 30±6 mg/day), 13 subjects with COPD without known diabetes admitted for other indications and not treated with prednisolone (Group 2, 8 men, age = 75±11 years) and 7 COPD patients with known diabetes treated with prednisolone (Group 3, 5 men, age = 84±9 years, prednisolone = 26±9 mg/day) underwent continuous glucose monitoring for up to 72 hours.

Significantly more subjects in Group 1 (21/40 (53%), p=0.02) and Group 3 (7/7 (100%), p=0.003) recorded a glucose of ≥11.1 mmol/L during continuous glucose monitoring than in Group 2 (1/13 (8%)). The mean glucose between 0.00-12.00 hours for Group 3 (7.9±2.0 mmol/L) was significantly greater than in the other two groups (p<0.005 for both comparisons), while mean glucose between 0.00-12.00 hours in Group 1 (6.2±1.2 mmol/L) and Group 2 (6.0±0.9 mmol/L) were not significantly different. In contrast, the mean glucose between 12.00-24.00 hours for Group 1 (7.9±1.4 mmol/L) and Group 3 (10.5±1.8 mmol/L) were both were significantly greater than Group 2 (6.5±0.8 mmol/L, p<0.05 for both comparisons).

In summary, prednisolone frequently caused hyperglycemia in hospitalized patients, predominantly in the afternoon and evening. Treatment of prednisolone-induced hyperglycemia should be targeted at this time of the day.

Acknowledgements: Supported by Foundation Daw Park, Flinders University, Novo Nordisk Regional Diabetes Scheme and Medtonic Minimed.

(1) Arnaldi G et al. Diagnosis and complications of Cushing's syndrome: A consensus statement. J Clin Endocrinol Metab 2003;88: 5593-601

256

PIGMENT EPITHELIUM DERIVED FACTOR AS A MARKER OF CARDIOMETABOLIC RISK FACTORS AND INSULIN RESISTANCE IN OVERWEIGHT WOMEN WITH AND WITHOUT POLYCYSTIC OVARY SYNDROME

A. E. Joham[1,2], H. J. Teede[1,2], S. K. Hutchison[1,2], N. K. Stepto[3,4], C. Harrison[2,3], M. J. Watt[3]

[1]Department of Diabetes, Southern Health, Clayton, VIC, Australia

[2]Jean Hailes Clinical Research Unit, The School of Public Health and Preventative Medicine, Monash University, Clayton, VIC, Australia

[3]Department of Physiology, Monash University, Clayton, VIC, Australia

[4]Centre for Aging, Rehabilitation, Exercise and Sport, Victoria University, Melbourne, VIC, Australia

Background : Pigment Epithelium-Derived Factor (PEDF) is a glycoprotein belonging to the superfamily of serine protease inhibitors. PEDF appears to play a causal role in insulin resistance (IR) in animal models and may contribute to IR in human IR states including obesity and type II diabetes. Polycystic Ovary Syndrome (PCOS) is a common IR state, associated with obesity and an increased risk of cardiometabolic sequelae including diabetes, hypertension and dyslipidaemia in women of reproductive age. Exploring the relationship between PEDF, IR and metabolic risk factors in PCOS, may improve the understanding of PEDF's role in IR and associated metabolic abnormalities.

Objectives : To compare PEDF levels in obese controls without PCOS and obese, IR, normoglycemic women with PCOS. To assess the relationship of PEDF and established metabolic risk factors.

Design : Cross-sectional study

Setting : Academic medical centre

Participants : 20 overweight women with PCOS (median age 28.0[26.0–35.0] years and median body mass index [BMI] 35.1kg/m^2,[32.0–39.7]) and 14 BMI matched control women without PCOS (median age 35.5 years,[32.0–38.0], median BMI 34.7kg/m^2,[32.7–37.3]).

Method : Following recruitment from community advertisement and screening, women were withdrawn from interfering medications and studied following a 3 month washout period. Blood samples were taken for PEDF and metabolic markers. Detailed body composition measures and gold standard euglycaemic hyperinsulinaemic clamps were performed.

Main Outcome Measures : Plasma levels of PEDF and glucose infusion rate (GIR) on clamp study.

Results : Mean GIR was lower [83 $mg.m^{-2}.min^{-1}$, p=0.01] in overweight women with PCOS compared to overweight controls [125 $mg.m^{-2}.min^{-1}$, p=0.01], yet PEDF levels were not different between groups. PEDF levels related to known metabolic risk factors (systolic [point estimate=0.13 (standard error=0.04), p=0.01] and diastolic blood pressure [0.19(0.07), p=0.01], lipid profile {HDL [-5.98(2.13), p=0.01], triglyceride levels [2.01(0.87), p=0.03]}) and abdominal visceral adiposity [0.04(0.01), p=0.01]. These relationships were independent of fat mass. Overall, plasma PEDF negatively related to GIR [-0.03(0.02), p=0.05], with no significant difference between the 2 groups on adjusting for fat mass [-0.03(0.02), p=0.08].

Conclusions : Plasma PEDF relates to IR and metabolic risk factors in overweight women with and without PCOS. PEDF shows promise as a marker of metabolic status and IR. It may be involved in the etiology of these metabolic disturbances. Given the current paucity of accurate and practically applicable markers of IR and the poor understanding of etiology of IR, PEDF may be of clinical importance.

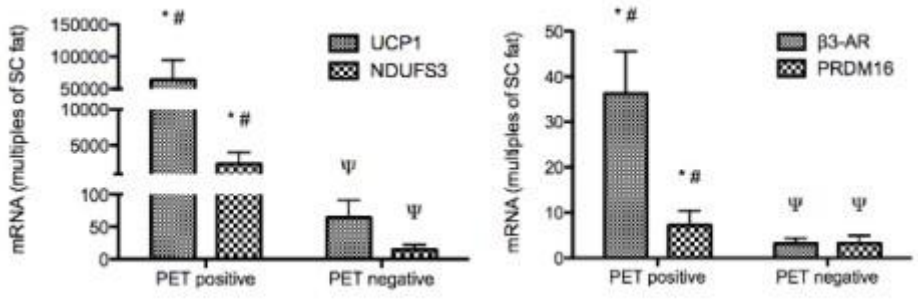
257

BROWN ADIPOSE TISSUE IS PRESENT IN THE MAJORITY OF ADULT HUMANS: HISTOLOGIC AND MOLECULAR CHARACTERIZATION OF PET POSITIVE AND NEGATIVE SUPRACLAVICULAR FAT

P. Lee[1,3], J. T. Zhao[1], M. Swarbrick[2], R. Bova[4], G. Gracie[5], J. Freund[6], J. R. Greenfield[2,3], K. K.Y. Ho[1,3]

[1]Pituitary Research Unit, Garvan Institute of Medical Research, Sydney, NSW, Australia
[2]Diabetes and Obesity Programme, Garvan Institute of Medical Research, Sydney, NSW, Australia
[3]Department of Endocrinology, St Vincent's Hospital, Sydney, NSW, Australia
[4]Department of Surgery, St Vincent's Hospital, Sydney, NSW, Australia
[5]Department of Anatomical Pathology, St Vincent's Hospital, Sydney, NSW, Australia
[6]Department of Nuclear Medicine, St Vincent's Hospital, Sydney, NSW, Australia

Positron Emission Tomography (PET)-CT has identified metabolically active fat in adult humans based on uptake of labelled glucose. This is localised most commonly in the supraclavicular (SCV) fossae, and confirmed to be brown adipose tissue (BAT) histologically. The report of a prevalence of only 5-10% is likely an underestimation because of poor sensitivity of PET-CT [1]. The aim of the present study is to determine whether BAT is present in PET negative SCV fat. We hypothesise BAT is present in most adult humans. Twelve patients who underwent pre-operative PET-CT for staging of head and neck malignancy were recruited. In patients with positive PET-CT scans (SUV of more than 2), open biopsies from the SCV fossae were obtained as delineated by PET-CT. In patients with negative PET-CT, fat biopsies were obtained from an identical region. Subcutaneous (SC) neck fat was obtained from all patients as negative control. Fat samples were processed for histological and molecular analyses. BAT was defined by the presence of multi-lobulated lipid droplets, UCP1 immunostaining and mRNA for UCP1, NDUFS3, PRDM16 and β3-adrenoceptor (AR). Transcripts were quantified by PCR and expressed as multiples over SC fat. PET-CT was positive in 3 and negative in 9 patients. By histology, PET positive fat harboured multi-lobulated lipid droplets and stained strongly for UCP1. SC fat did not show any cells with multi-lobulated droplets or with UCP1 staining. By contrast, PET negative fat demonstrated a predominance of cells with unilobulated lipid droplets, with scattered cells containing multi-lobulated lipid droplets and variable UCP1 staining. Molecular analyses of fat biopsies showed lower but clear expression of UCP1, NDUFS3, β3-AR and PRDM16 transcripts [Figure]. In summary PET negative SCV fat, but not SC fat, contains brown adipocytes, at much lower abundance than PET positive SCV fat. We conclude that BAT is present in the supraclavicular fossae of majority of humans. PET avidity is determined by the abundance of BAT.



*p<0.01 compared to PET negative fat, #p<0.001 compared to SC fat, Ψp<0.01 compared to SC fat.

(1) Lee P, Ho KKY, Fulham MJ. Importance of brown adipose tissue. NEJM 2009; 361: 4181

258

UNILATERAL ADRENALECTOMY IMPROVES URINARY PROTEIN EXCRETION BUT DOES NOT ABOLISH ITS RELATIOSHIP TO SODIUM EXCRETION IN PATIENTS WITH ALDOSTERONE-PRODUCING ADENOMA

E. Pimenta, R. D. Gordon, A. H. Ahmed, D. Robson, D. Cowley, C. Kogovsek, M. Stowasser

Endocrine Hypertension Research Centre, University of Queensland Medical School, Woolloongabba, QLD, Australia

Background: Experimental and human data suggest that adverse cardiovascular and renal effects of aldosterone excess are dependent upon concomitant dietary salt intake. Increased urinary protein (Uprot) is an early sign of nephropathy independently associated with cardiovascular risk. We have previously reported a positive association between Uprot and urinary sodium (UNa) in patients with hyperaldosteronism, but not in patients with normal aldosterone levels.

Objective: We aimed to determine if Uprot is related to UNa in patients with aldosterone-producing adenoma (APA) and whether the degree of Uprot and strength of this relationship is reduced following correction of hyperaldosteronism.

Methods: Subjects with APA (n=24) underwent measurement of 24h Uprot and UNa before and after unilateral adrenalectomy (follow-up 15.0±11.9 months).

Results: Following surgery, mean clinic systolic blood pressure fell (150.4±18.2 vs. 134.5±14.5 mmHg, p=0.0008), despite a reduction in number of antihypertensive medications, and Uprot (211.2±101.6 vs. 106.0±41.8 mg/day, p<0.0001) decreased. There was a positive correlation between Uprot and UNa both before (r=0.5477, p=0.0056) and after (r=0.5097, p=0.0109) adrenalectomy. Changes in urinary sodium independently predicted Uprot reduction (p=0.0189).

Conclusions: These findings suggest that both aldosterone levels and dietary salt contribute to renal damage, and that once glomerular damage occurs and is not completely resolved following correction of hyperaldosteronism, it leads to perpetuation of the positive relationship between dietary salt and proteinuria. If confirmed, our study supports treatment strategies based on reduction of aldosterone

effects, by adrenalectomy or mineralocorticoid receptor blockade, in conjunction with low-salt diet to provide additional target organ protection in patients with hyperaldosteronism.

259

EFFECT OF ATENOLOL ON ALDOSTERONE/RENIN RATIO CALCULATED BY BOTH PLASMA RENIN ACTIVITY AND DIRECT RENIN CONCENTRATION IN HEALTHY MALE VOLUNTEERS

A. H. Ahmed[1], R. D. Gordon[1], P. J. Taylor[1], G. Ward[2], E. Pimenta[1], M. Stowasser[1]

[1]Endocrine Hypertension Research Centre, Princess Alexandra Hospital, Woolloongabba, QLD, Australia
[2]Endocrinology, Sullivan & Nicolaides Pathology, QLD, Australia

Background: The most popular screening test for primary aldosteronism (PAL) is the plasma aldosterone/ renin ratio (ARR). Medications, dietary sodium, posture and time of day all affect renin and aldosterone levels, and can result in false negative or positive ARRs if not controlled. Opinions are divided on whether beta-adrenoceptor blockers significantly affect the ARR.

Methods: Normotensive, non-medicated male volunteers (n=21) underwent measurement (seated, midmorning) of plasma aldosterone (by HPLC-tandem mass spectrometry), direct renin concentration (DRC), renin activity (PRA), cortisol, electrolytes and creatinine and urinary aldosterone, cortisol, electrolytes and creatinine at baseline, and after one week (25mg daily) and four weeks (50mg daily for three additional weeks) of atenolol.

Results: Compared with baseline, levels of aldosterone, DRC and PRA were lower ($p<0.001$) after both one and four weeks [baseline median 189 (range 107-449) pmol/L, 40 (20-53) mU/L, and 4.6 (1.2-6.8) ng/ml/hr; one week 166 (75-424) pmol/L, 34 (15-45) mU/L, and 2.6 (1.0-5.4) ng/ml/hr; four weeks 136 (73-394) pmol/L, 16 (10-40) mU/L and 2.1(0.8-3.8) ng/ml/hr respectively]. ARR was significantly higher after one week compared with baseline when calculated using PRA [61 (21-161) vs 65 (20-213), $P<0.01$] but not DRC [5 (4-10) vs 5 (4-11)]. At four weeks, ARR calculated by both PRA [78 (41-182)] and DRC [8 (4-31)] were significantly higher ($p<0.001$) compared to baseline. Cortisol levels were significantly lower [92 (47-123) vs 66 (39-119) ng/ml, $p<0.01$]. There were no changes in plasma sodium, potassium, creatinine or any urinary measurements.

Conclusion: Beta-blockers can significantly raise the ARR and thereby increase the risk of false positives during screening for PAL.

260

OVERLAPPING FUNCTIONAL EFFECTS OF PROGESTERONE IN MOUSE AND HUMAN MAMMARY GLAND HAVE DISTINCT TRANSCRIPTIONAL SIGNATURES

J. D. Graham[1], J. P. Lydon[2], C. L. Clarke[1]

[1]Westmead Institute for Cancer Research, University of Sydney at Westmead Millennium Institute, Westmead, NSW, Australia
[2]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas 77030, United States

Progesterone (P) exerts fundamental control over the female reproductive system. In the human breast the functions regulated by P remain largely unexplored, and, although P increases proliferation in the mammary gland of both species, the overlap between P action in human and mouse is unknown.

To identify whether P regulates proliferation in the human and mouse mammary gland by overlapping mechanisms, we explored gene expression profiles of P-treated human and mouse mammary epithelium at a range of times (6h, 48h in human, 4h, 16h, 28h, 76h in mouse). Functional annotation analysis revealed overlaps in proliferation categories, but with different timing of their appearance. In the human breast, within 6h, there was a marked enrichment of transcripts involved in DNA replication licensing and cell cycle, followed by increased BrdU incorporation 42h later. By contrast, functional categories enriched at early time points in the mouse included adhesion, extracellular signalling and development, reflecting profound tissue remodelling. At 76h treatment, there was increased proliferation in the mouse, and the up-regulation of transcripts associated with DNA replication and cell cycle, suggesting that in the rodent mammary gland, reorganization of the extracellular environment occurs prior to proliferation.

Direct comparison of proliferation-related genes in mouse and human revealed that the actual genes regulated by P were largely different, with less than 10% of identical genes being regulated in both species. The greatest overlap occurred in the cell cycle progression and DNA replication licensing transcripts, which were increased by P early in the human model and at 76h in the mouse.

Taken together, this study shows that P drives largely non-overlapping programs of gene expression in the mouse mammary gland and human breast, and that distinct higher-level mechanisms may govern a common proliferative endpoint.

261

ANDROGEN RECEPTOR DNA BINDING IS NOT REQUIRED FOR NORMAL MAMMARY GLAND STRUCTURE AND FUNCTION IN MICE

Y. Gao, U. Simanainen, K. A. Walters, D. J. Handelsman

Andrology, Anzac Research Institute, Concord, NSW, Australia

Androgen receptor (AR) is expressed in normal developing and mature mammary cells of women and mice [1], indicating a likely role for mammary growth and function [2]. AR knockout (ARKO) females with large AR deletions lacking both DNA- and ligand binding domains, have retarded breast development with reduced branching, decreased lobuloalveolar development, and fewer milk-producing alveoli in the lactating glands [3]. We used our novel ARKO mouse model with an in-frame deletion of exon 3 of the AR gene (ARKOEx3;Cre-LoxP) leading to the production of a non-functional AR protein just lacking the 2nd zinc finger and thereby DNA binding ability (4). Structural, functional and molecular changes in 4th mammary glands were compared between virgin, sexually mature wild-type (WT) and ARKO females. The ARKOEx3 mammary glands displayed normal structural development as analyzed by whole mount staining of the mammary epithelia at estrus and during pregnancy. The ductal distance (center of the lymph node to the distal end of the terminal end buds) did not differ at estrus (150±11 [mean±SD] vs160±15 mm) between virgin ARKO and WT females or in pregnant ARKO and WT (168±7 vs 175±9 mm). Mammary gland function was unaffected with normal pup survival and pup growth over 30 days. Normal mammary gland development and function was further supported by unchanged progesterone receptor and estrogen receptor α protein (stereology) and mRNA levels (realtime RT-PCR) in ARKO females. Epithelial proliferation quantified by PCNA immunopositivity (stereology) was non-significantly increased (p=0.092) by 20% in ARKO female mammary glands compared to WT. In conclusion, we show that contrary to ARKO mice with major deletions of AR, minimally truncated AR lacking only DNA binding avoids secondary consequences of disrupting co-regulator or other post-receptor effects allowing normal structural or functional development of the mammary gland.

(1) Dimitrakakis C et al., Fertil Steril 77, 2002
(2) Liao DJ et al., J Steroid Biochem Mol Biol 80, 2002
(3) Yeh et al., J Exp Med 198, 2003
(4) Walters et al, Human Reprod Update, 2010

262

A NEW FUNCTION FOR THE FUSOGENIC ENDOGENOUS RETROVIRAL ENVELOPE PROTEIN SYNCYTIN-1: ASSESSMENT OF ITS IMMUNOSUPPRESSIVE PROPERTIES

J. M. Tolosa[1,2], J. E. Schjenken[1], V. L. Clifton[3], R. Smith[1]

[1]Mothers and Babies research Centre, The University of Newcastle, Newcastle, Australia
[2]Facultad de Ciencias medicas, Universidad de Santiago de Chile, Santiago, Chile
[3]Department of Paediatrics and Reproductive Health, University of Adelaide, Adelaide, SA, Australia

Introduction: The human placenta expresses endogenous retroviral envelope proteins. One of them, syncytin-1 is highly expressed in the syncytiotrophoblast and is thought to be a key factor in regulating syncytialisation due to its fusogenic properties. Many retroviral envelope proteins are immunosuppressive due to the presence of a highly conserved sequence called the Immunosuppressive Domain (ISD). Syncytin-1 carries a sequence homologous to the consensus ISD. In light of this information, we hypothesised that syncytin-1 has, in addition to its fusogenic function, immunosuppressive properties and may therefore play a role in maternal immune tolerance. Methods: A synthetic peptide and the recombinant ectodomain of syncytin-1 which both carry the ISD were produced and used in this study. The recombinant ectodomain of syncytin-1 was purified using a combination of affinity chromatography and gel filtration. The synthetic peptide and the recombinant ectodomain of syncytin-1 were tested in vitro using human whole blood/PBMC cultures challenged with LPS or PHA. Supernatans were assayed for the production of TNF-α, IFN-γ and CXCL10 using commercial ELISA kits. Results: Both the synthetic peptide and syncytin-1 recombinant protein inhibited the release of TNF-α by human blood cells in a dose dependent manner following maximun stimulating doses of LPS (10μg/ml). At 1μM, syncytin-1 recombinant ectodomain inhibited, in whole human blood, the release of the inflammatory cytokine TNF-α (50%) and the release of the chemokine CXCL10 (65%) which is involved in Th1 immune responses and allograft rejection. Syncytin-1 also inhibited the release of IFN-γ by 30% in PHA stimulated PBMC at a concentration of 1μM.

Discussion: Retroviral envelope proteins have previously been shown to inhibit Th1 immune responses and induce Th2 response. Here we show experimental evidence in favour of syncytin-1 having an immunosuppressive role. This is a novel role for syncytin-1 and suggests that syncytin-1 may be relevant in maternal immune tolerance towards feto-placental antigens.

263

TUMOUR NECROSIS FACTOR-A STIMULATES HUMAN NEUTROPHILS TO RELEASE PRE-FORMED ACTIVIN A

Y. Chen[1,2], H. Wu[1], W. R. Winnall[1], K. L. Loveland[3], D. J. Phillips[4], J. A. Smith[2], M. P. Hedger[1]

[1]Monash Institute of Medical Research, Clayton, VIC, Australia
[2]Department of Sugery (MMC), Monash University, Clayton, VIC, Australia

[3]Department of Biochemistry, Monash University, Clayton, VIC, Australia
[4]Research Services, La Trobe University, VIC, Australia

Activin A, a member of the transforming growth factor-β superfamily, was first described for its ability to stimulate FSH release by the pituitary. It has since been shown in animal models of lipopolysaccharide (LPS)-induced endotoxaemia to be a critical early inflammatory cytokine released into the circulation at the same time as tumour necrosis factor (TNF)-α. The source of circulating activin following LPS treatment has not been identified, hence the potential contribution of leukocyte subsets was examined. Human neutrophils were isolated from healthy volunteers using Ficoll gradient separation and cultured in RPMI 1640 without serum. Activin A protein and mRNA was measured by ELISA and qRT-PCR, respectively. Activin A immunocytochemistry was performed on blood cell cytospin preparations. Isolated human neutrophil lysates contained 20-fold higher levels of pre-formed activin A than mononuclear cells (monocytes and lymphocytes). Activin was predominantly immunolocalized in the cytoplasm of neutrophils. TNF-α, but not LPS itself, stimulated the release of activin A from purified neutrophils within 1h, but activin mRNA expression did not increase until 12h of culture. The amount of activin released following TNF-α stimulation did not change between 1h and 12h, suggesting that only pre-formed activin was released. Specific inhibition of the p38 MAP kinase signaling pathway prevented the effect of TNF-α on activin release. TNF-α also induced neutrophil apoptosis, but the release of activin preceded cell death, indicating that the release was not simply due to lysis. These data provide the first evidence that neutrophils are a major source of pre-formed activin and may be an important contributor to the early peak in serum activin following an LPS challenge *in vivo*. Since activin plays multiple roles in controlling reproduction, the rapid release of activin by neutrophils during inflammation may contribute to the disturbances of fertility, endocrine function and pregnancy that are associated with systemic inflammation.

264

EARLY GROWTH RESPONSE TRANSCRIPTION FACTORS STIMULATE CYP19A1 EXPRESSION IN RESPONSE TO TNFA – NOVEL MECHANISMS OF OESTROGEN BIOSYNTHESIS

S. Q. To[1,2], K. C. Knower[1], E. R. Simpson[1,2], C. D. Clyne[1,2]

[1]Prince Henry's Institute, Clayton, VIC, Australia
[2]Biochemistry and Molecular Biology, Monash University, Clayton, VIC, Australia

Breast cancer remains the leading cause of cancer-related death in Australian women. In post-menopausal cases, up to 70% of tumours are classed as oestrogen receptor positive (ER+), dependent on oestrogen for continued growth and proliferative advantage. Adjuvant anti-oestrogen therapies are considered the cornerstone approach to the treatment of such tumours, and research is ongoing to maximize the effectiveness of drug treatments.

The major source of oestrogens for growth of ER+ breast cancers is local conversion of androgen precursors by the enzyme P450 aromatase. Inflammatory factors such as Tumour Necrosis Factor-α (TNFα) stimulate transcription of the CYP19A1 gene that encodes aromatase, via its adipose-specific promoter I.4 (PI.4). The pathways by which this is achieved are not fully understood. This study aims to identify the mechanisms underlying TNFα-dependent aromatase induction in the context of oestrogen-dependent breast cancer. Primary human breast adipose fibroblasts (BAFs) were treated with TNFα and potential regulatory factors of PI.4 were assessed for changes in mRNA expression through the use of a custom array. The family of Early Growth Response genes Egr1, Egr2, Egr3 and Egr4 were all found to be significantly upregulated in a time-dependent manner in response to TNFα, as validated by qRT-PCR. Egr2, Egr3 and Egr4 were able to induce PI.4-driven luciferase reporter activity, and 5' deletion analysis showed this was mediated via an overlapping Sp1/Egr binding site within the promoter region. Mutagenesis of this binding site and ChIP analysis will further elucidate the nature of Egr-mediated PI.4 activity in response to TNFα.

Understanding TNFα signalling and the transcription factors it activates to turn on PI.4 CYP19A1 expression in BAFs is vital to the understanding of breast cancer pathology. These insights may help in the development diagnostic tools or novel therapeutics in order to help tackle the growing instances of this disease.

265

EPIGENETIC REGULATION OF PROGESTERONE RECEPTOR ISOFORM EXPRESSION IN TERM HUMAN MYOMETRIUM

S. Chai[1,2], R. Smith[1,2], G. Madsen[1,2]

[1]Mothers and Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia
[2]School of Medicine and Public Health, The University of Newcastle, Newcastle, NSW, Australia

In most mammals, progesterone withdrawal initiates parturition. In humans, progesterone withdrawal occurs by a decrease in myometrial progesterone responsiveness (functional progesterone withdrawal), achieved by increased expression of progesterone receptor-A (PR-A), a repressor of progesterone actions mediated through the active progesterone receptor-B (PR-B). Both isoforms are encoded by a single gene but independently regulated by two distinct promoters. As the extent of progesterone responsiveness is inversely related to the amount of PR-A relative to PR-B, it is crucial to understand how the expression of the two isoforms is regulated. One possibility is that regulation occurs through epigenetic changes such as, histone acetylation or methylation, in the PR gene. Using chromatin immunoprecipitation (ChIP), we investigated the presence of acetylated histones H3 (aH3) and H4 (aH4), and trimethylated-H3-lysine4 (H3K4me3), which are epigenetic marks for active gene expression, across the promoter regions of PR in term human myometrium. The occupancy of RNA polymerase-II (Pol-II) was also determined, as an indicator of transcriptional activity. Myometrial samples were collected from women

undergoing caesarean deliveries at term, fixed with formaldehyde and sonicated to reduce DNA fragment size, and then immunoprecipitated with antibodies against aH3, aH4, H3K4me3 and Pol-II. The cross-links were reversed, and purified DNA were analysed by real time PCR using primers specifically designed to span the PR-A and PR-B promoters. Our results showed higher activities of H3 and H4 acetylation and H3K4 trimethylation along the promoter regions of PR-A compared to that of PR-B. Concurrently, there was also increased Pol-II occupancy on the PR-A promoter. These data are in keeping with an increase in myometrial expression of PR-A at term, consistent with a functional withdrawal of progesterone. Thus, myometrial expression of PR-A and PR-B is determined by the degree of acetylation and/or methylation of histones bound to their respective promoters.

266

CHRONIC ICV INFUSION OF OCRF ELIMINATES THE CORTICOSTERONE DIURNAL RHYTHM WHILE MAINTAINING PULSATILITY

H. C. Atkinson[1,2], S. A. Wood[1], E. S. Castrique[1], Y. M. Kershaw[1], S. L. Lightman[1]

[1]Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology, University of Bristol, Bristol, United Kingdom

[2]School of Women's and Infants' Health, University of Western Australia, Perth, WA, Australia

The hypothalamic-pituitary-adrenal (HPA) axis displays both circadian and ultradian rhythms of corticosteroid secretion. This study examined the effect of a constant (ie non-rhythmic) source of the corticotrophin releasing factor (CRF) on HPA axis rhythmicity.

Male rats received a chronic infusion of vehicle or oCRF (24 µg/day; donated by Wylie Vale) into the left lateral ventricle for 6 days. On the final day blood samples were collected every 10 minutes for 24 hours and in the hour following a mild noise stress. Brains were collected and the amount of POMC mRNA in the pituitary, CRF mRNA in the hypothalamus, and AVP mRNA in the hypothalamus were determined by in-situ hybridisation.

Males receiving a constant infusion of vehicle displayed a normal diurnal profile of corticosterone with a robust response to the white noise stress. By comparison, the oCRF treated males did not display diurnal variation in corticosterone concentrations although pulsatility was maintained. Furthermore, there was no obvious corticosterone response to noise stress.

Constant infusion of oCRF had marked effects on the neuroendocrine pathways in the brain and pituitary when compared to controls (t-tests). The expression of CRF in the PVN of the hypothalamus was decreased ($P<0.001$) to such a degree that the outline of the PVN was only discernable in one animal treated with oCRF. oCRF treatment also resulted in a 20 % reduction ($P<0.05$) in AVP expression in the PVN and a 70% increase ($P<0.01$) in POMC expression in the anterior pituitary. There was significant atrophy of the thymus in the oCRF treated rats with thymus weights 40% ($P<0.001$) lower than controls. There was no effect of oCRF treatment on GR expression in the anterior pituitary.

Constant infusion of exogenous oCRF resulted in a powerful inhibition of endogenous CRF expression and although it inhibited the diurnal pattern of circulating corticosterone it did not disrupt underlying pulsatility.

267

INTERACTION BETWEEN TESTOSTERONE AND GROWTH HORMONE ON WHOLE BODY PROTEIN ANABOLISM IS MEDIATED THROUGH THE LIVER

V. Birzniece[1], U. J. Meinhardt[1], D. J. Handelsman[2], K. K.Y. Ho[1]

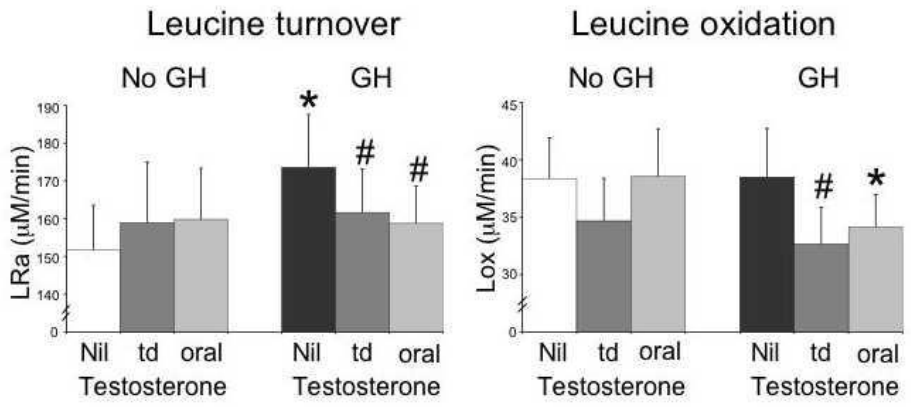
[1]Pituitary Research Unit and Department of Endocrinology, Garvan Institute of Medical Research, St Vincent's Hospital, Darlinghurst, NSW, Australia

[2]ANZAC Research Institute, Concord Hospital, Sydney, NSW, Australia

Growth hormone (GH) and testosterone (T) both exert protein anabolic effect and may act synergistically. Liver and muscle are major sites where protein metabolism is regulated. We aimed to determine whether the site of GH and T interaction is primarily hepatic or extrahepatic. We therefore compared the impact on whole body protein metabolism of T administered via oral route (solely hepatic T exposure) with transdermal T replacement (systemic T exposure) in the presence or absence of GH.

Fifteen hypopituitary men with GH and T deficiencies participated in a randomised open-label crossover study of 2-wk treatments with transdermal T (10 mg) and oral T (40 mg), with or without GH replacement (0.6 mg/day) for at least 3 months. The dose of T administered orally achieves physiological portal T concentrations without spill-over into the systemic circulation (Clin Endocrinol 2009; 71:715-21). Whole body leucine turnover was measured, from which leucine rate of appearance (LRa; an index of protein breakdown) and leucine oxidation (Lox; an inverse measure of anabolism) was estimated at the end of each treatment period.

In the absence of GH, neither transdermal nor oral T affected LRa. GH therapy significantly increased LRa, which was reduced by T treatment with the effect not different between transdermal and oral T administration. In the absence of GH, neither transdermal nor oral T affected Lox. Treatment with GH did not significantly affect Lox. However addition of T treatment reduced Lox, with the effect not significantly different between transdermal and oral T.



$p<0.05$ * vs no treatment, # vs GH only

In summary, leucine turnover was not affected by T but stimulated by GH, an effect abrogated with co-administration of T. Leucine oxidation was reduced by combined administration of GH and T but not by either hormone alone. The effects of T, whether administered by an oral or transdermal route, were similar.

We conclude, in the doses used, T alone did not affect protein metabolism. In the presence of GH, T stimulates anabolism by reducing protein breakdown and oxidation. Because there was no difference between systemic and solely hepatic effects of T, the liver is primary site of the GH and T interaction on whole protein metabolism.

133

LESION WEIGHT AND GLANDULAR DEVELOPMENT ARE SUPPRESSED IN A TGFB1 DEFICIENT MOUSE MODEL OF ENDOMETRIOSIS

M. Z. Johan, W. V. Ingman, S. A. Robertson, M. L. Hull

Obstetrics and Gynaecology, Research Centre for Reproductive Health, Adelaide, SA, Australia

Endometriosis causes subfertility, pelvic pain and dysmenorrhea, and affects 10% of women of reproductive age globally. The pathology of endometriosis is still poorly understood; however microarray data from a mouse model revealed transforming growth factor beta 1 (TGFB1) as central component in molecular pathways that promote tissue remodelling of ectopic endometrial tissues [1]. We hypothesised that a host deficiency of TGFB inhibits the growth of endometriotic lesions and changes the cellular composition of the tissues.

To test this hypothesis, human eutopic endometrial tissue was implanted into *Tgfb1 -/-* mice with *Tgfb1+/+* wildtype mice were used as controls (n=8 and 19 respectively). All mice were on a background of severe combined immunodeficiency to prevent graft rejection. The weight and volume fraction of the glandular and stromal compartments of the resulting lesions were evaluated and the sections were stained with BrdU as a marker of proliferating cells.

Sixty percent of mice developed ectopic endometrial lesions in both groups. The median weight of the xenografts from *Tgfb1+/+* wildtype mice was 11-fold higher than the *Tgfb1-/-* mice (Mann-Whitney U test, $p = 0.0275$). The glandular volume fraction in endometriosis-like lesion from *Tgfb1-/-* mice was 0.35 and was 33% lower than in lesions from the control mice (volume fraction of glands = 0.52) (independent t test, $p = 0.0415$).

These studies show that TGFB1 is critical for normal endometriosis-like lesion development and a host deficiency of TGFB1 is associated with reduced weight and glandular volume fraction of xenografts. Targeted suppression of TGFB1 in the host response could be a successful therapeutic strategy for women with this disease.

(1) Hull, M.L., et al., Endometrial-Peritoneal Interactions during Endometriotic Lesion Establishment. Am J Pathol, 2008. 173(3): p. 700-715.

134

DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS TO MULLERIAN TISSUE

L. YE[1], R. Mayberry[2], E. Stanley[2], A. Elefanty[2], C. Gargett[1]

[1]O&G, MIMR, Monash University, Melbourne, VIC, Australia
[2]MISCL, Monash University, Melbourne, VIC, Australia

The human uterus develops from the distal Mullerian Duct, a derivative of the mesoderm germ layer. Unlike other mammalian species (eg. mouse) the endometrium of the human uterus develops prenatally during gestation. Little is known about the developmental process involved. A better understanding of human endometrial development may shed light on the mechanisms involved in endometrial regeneration and pathogenesis of adult proliferative endometrial diseases. Mouse neonatal uterine mesenchyme (mNUM) is inductive and can maintain the phenotype of normal adult human endometrial epithelial cells [1] . Both adult human endometrial stroma and neonatal mouse endometrial mesenchyme secrete growth factors of the TGF-beta family including BMPs which have been shown to play an important role in differentiation of human embryonic stem cells (HESC) [2, 3] . Hypothesis: mNUM will direct differentiation of HESC to form Mullerian Duct-like epithelium. Aim: to investigate the role of mNUM in differentiating HESC *in vitro* and *in vivo* using A tissue recombination technique. Method: Embryoid bodies (EB) were formed from GFP labelled HESC (ENVY) and GFP-MIXL1 HESC reporter line [4, 5] and recombined with 2x0.5mm pieces of day 1 epithelial cell-free mNUM. Recombinant tissues were either harvested for gene expression analysis or grafted under the kidney capsule of NOD/SCID mice. Results: We found by qRT-PCR that mNUM induces HESC to form mesendoderm/mesoderm progenitors *in vitro*, obligate intermediates of the developing Mullerian Duct. After further incubation in vivo under the guidance of mNUM, HESC differentiated to form duct-like structures comprising mesoepithelial cells that co-expressed several key developmental proteins of the Mullerian Duct including Emx2, Pax2, Hoxa10, CA125, and also intermediate filament markers such as CK8/18, Vimentin, (n=8). Conclusion: Our study demonstrated for the first time that mNUM can direct HESC to form a mesodermally derived epithelium that is Mullerian Duct-like, providing a novel model for studying human uterine development.

(1) 1. Kurita, T., et al., The activation function-1 domain of estrogen receptor alpha in uterine stromal cells is required for mouse but not human uterine epithelial response to estrogen. Differentiation

(2) 2. Hu, J., C.A. Gray, and T.E. Spencer, Gene expression profiling of neonatal mouse uterine development. Biol Reprod, 2004. 70(6): p. 1870-6.

(3) 3. Stoikos, C.J., et al., A distinct cohort of the TGFbeta superfamily members expressed in human endometrium regulate decidualization. Hum Reprod, 2008. 23(6): p. 1447-56.

(4) 4. Davis, R.P., et al., Targeting a GFP reporter gene to the MIXL1 locus of human embryonic stem cells identifies human primitive streak-like cells and enables isolation of primitive hematopoietic pre

(5) 5. Costa, M., et al., The hESC line Envy expresses high levels of GFP in all differentiated progeny. Nat Methods, 2005. 2(4): p. 259-60.

135

GENOME-WIDE ASSOCIATION STUDY IDENTIFIES A LOCUS AT 7P15.2 ASSOCIATED WITH THE DEVELOPMENT OF MODERATE-SEVERE ENDOMETRIOSIS

G. W. Montgomery[1], J. N. Painter[1], C. A. Anderson[2,3], D. R. Nyholt[1], S. Macgregor[1], S. H. Lee[1], P. M. Visscher[1], P. Kraft[4,6], N. G. Martin[1], A. P. Morris[2], S. A. Treloar[1,5], S. H. Kennedy[7], S. A. Missmer[4,6], K. T. Zondervan[2,7]

[1]Queensland Institute of Medical Research, Brisbane, QLD, Australia
[2]Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom
[3]Wellcome Trust Sanger Institute, Hinxton, United Kingdom
[4]Harvard School of Public Health, Boston, MA, United States
[5]Centre for Military and Veterans' Health, University of Queensland, Bsribane, QLD, Australia
[6]Brigham and Women's Hospital, Boston, MA, United States
[7]Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Oxford, United Kingdom

Endometriosis is a common gynaecological disease associated with severe pelvic pain and sub-fertility. There is considerable debate whether different endometriosis stages represent disease progression, or whether moderate-severe (rAFS III/IV) disease is pathological and minimal-mild (rAFS I/II) an epiphenomenon. We conducted a genome-wide assocaition study using 540,082 SNPs in 3,194 surgically confirmed endometriosis cases and 7,060 controls from Australia and the UK. We used novel statistical methods to estimate the proportion of common variation explained by all markers and performed polygenic predictive modelling for disease stage, both showing significantly increased genetic loading among the 42% of cases with moderate-severe endometriosis. The strongest signals of association were also observed for moderate-severe disease. We subsequently genotyped 72 SNPs in an independent US dataset comprising 2,392 endometriosis cases and 1,646 controls. An association with rs7798431 on 7p15.2 for moderate-severe endometriosis ($P = 6.0 \times 10^{-8}$, OR = 1.34 (1.21-1.49)) was replicated, reaching combined genome-wide significance ($P = 1.7 \times 10^{-9}$; OR = 1.26 (1.17-1.35)). The implicated inter-genic region involves a 48 kb segment of high LD upstream of plausible candidate genes *NFE2L3* and *HOXA10*. This locus is the first to be robustly implicated in the aetiology of endometriosis, with evidence of association limited to moderate-severe disease.

136

PROGESTERONE RECEPTOR-REGULATED GENES IN THE PREOVULATORY OVARIAN FOLLICLE AND OVIDUCT

L. Akison, D. Russell, R. Robker

The Robinson Institute, Research Centre for Reproductive Health, University of Adelaide, Adelaide, SA, Australia

Ovulation is a highly regulated and precisely timed reproductive process but the underlying molecular mechanisms are not well understood. Progesterone receptor (PGR) is a transcription factor highly yet transiently expressed in granulosa cells (GCs) of preovulatory follicles; has low expression in cumulus-oocyte-complexes (COCs); and is abundantly expressed in the oviduct. $PR^{-/-}$ mice validate its essential role in ovulation as they are anovulatory, despite normal growth and development of ovarian follicles and oocytes. Our aim was to use microarray to identify differentially expressed genes in GCs, COCs and oviducts from $PR^{-/-}$ and $PR^{+/-}$ mice, specifically genes potentially involved in oocyte release and transport. GCs, COCs and oviducts were collected from 21d-old mice (n=5; 3 mice/replicate) at 8h post-hCG/44h post-eCG. Extracted RNA samples were hybridized to Affymetrix Mouse Gene 1.0 ST Arrays and post-experiment processing/analysis performed using Partek Genomics Suite. Gene ontology analysis was performed using Ingenuity Pathway Analysis (IPA). In GCs, 296 genes were differentially expressed ($P<0.05$); 78% down-regulated in $PR^{-/-}$. IPA identified genes involved in cancer migration/invasion, chemotaxis, and adhesion; the chemokine receptor *Cxcr4*, was >3-fold down-regulated in $PR^{-/-}$. Proteases were also decreased; *Adam8* (3.5-fold) and *Adamts1* (2.6-fold) in $PR^{-/-}$. In oviducts, 1003 genes were differentially expressed at $P<0.05$ and 266 genes at $P<0.01$; 93% were down-regulated in $PR^{-/-}$. IPA identified genes involved in cell adhesion, movement/migration, invasion and chemotaxis as well as muscle contraction and vasoconstriction. The most highly down-regulated was *Itga8* (>9-fold), one of 11 integrins, well known cellular adhesion receptors, differentially expressed. In COCs, 44 genes were differentially expressed ($P<0.05$); 52% down-regulated in $PR^{-/-}$. IPA identified 18 genes (41%) involved in cancer invasion/migration or adhesion. Thus, this study has identified novel gene targets for PGR regulation, which may have essential roles in the molecular control of oocyte release into the oviduct at ovulation.

167

MUSASHI FAMILY OF RNA BINDING PROTEINS: CELL CYCLE REGULATORS IN SPERMATOGENESIS

E. A. McLaughlin[1,3], B. A. Fraser[1,3], V. Pye[1,3], M. Bigland[1,3], N. A. Siddall[2,3], G. R. Hime[2,3]

[1]Environmental & Life Sciences, University of Newcastle, Callaghan, NSW, Australia
[2]Anatomy and Developmental Biology, University of Melbourne, Parkville, VIC, Australia
[3]ARC Centre of Excellence in Biotechnology & Development, Australia

Mammalian meiosis is a tightly regulated process involving specialized cell cycle progression and morphogenetic changes. We have demonstrated that the Musashi family of RNA binding proteins is implicated in the regulation of spermatogonial stem self renewal and germ cell differentiation. Here we describe the novel mechanism by which the Musashi family proteins, Msi1 and Msi2, act to control exit from spermatogonial mitotic amplification and normal entry into meiosis. Gene and protein analysis indicated overlapping Msi1 and Msi2

profiles in enriched populations of isolated germ cells and reciprocal subcellular expression patterns in spermatogonia and pachytene spermatocytes/ round spermatids in testes sections. Recombinant Msi1 protein-RNA pulldown and microarray analysis coupled with *in vitro* shRNA knockdown studies in spermatogonial culture and subsequent immunoprecipitation and qPCR established that Msi1 targeted Msi2 mRNA for post transcriptional translational repression. Immunoprecipitation of Msi2 target mRNA and subsequent qPCR together with *in vitro* shRNA knockdown studies in round spermatid culture identified a cell cycle inhibitor protein CDKN1C ($p57^{kip2}$) as the principal target of Msi2 translational inhibition. Immunolocalisation of CDKN1C protein indicated that expression of this cell cycle regulator coincided with the nuclear import of Msi1 and the appearance of cytoplasmic Msi2 expression in early pachytene spermatocytes. Using a transgenic Msi1 overexpression mouse model in conjunction with quantitative gene and protein expression, we confirmed Msi1 targeting of Msi2 and subsequent Msi2 targeting of CDKN1C for translational repression *in vivo.* Ectopic overexpression of Msi1 in germ cells induces substantial Msi2 downregulation and aberrant CDKN1C expression, resulting in abnormal spermatogenic differentiation, germ cell apoptosis/arrest and sterility. In conclusion, our results indicate a sophisticated molecular switch encompassing cell cycle protein regulation by Musashi family proteins, is required for normal exit from mitotic division, entry into meiosis and post meiotic germ cell differentiation.

168

TGFB 2-BETAGLYCAN REGULATE FOETAL TESTIS DEVELOPMENT *IN VITRO*

M. A. Sarraj, A. Umbers, J. K. Findlay, K. L. Stenvers

Reproductive Development, Prince Henry's Institute, Clayton, VIC, Australia

Betaglycan is a co-receptor for the TGFβ superfamily, known to modulate TGFβ binding in target cells. We have previously found that betaglycan null murine testes at 12.5-13.5 dpc display poorly delineated seminiferous cords and disrupted Leydig cell development (1). Both TGFβs and inhibins are expressed by the fetal testis and it is currently unclear which regulate its development. We tested the hypothesis that loss of betaglycan compromises the functions of TGFβ2 in the differentiating fetal testis as TGFβ2 is known to bind poorly to its type II receptor in the absence of betaglycan. We tested the effect of TGFβ2 on betaglycan wildtype and null foetal gonad/mesonephros complexes using hanging drop or agar block culture methods. From each embryo, one gonad acted as a control; the other was treated. Gonads were cultured in the presence or absence of TGFβ2 (2.5-5 ng/ml) for 48 hours (n =3 pairs). In both culture methods, development in the absence of exogenous growth factor recapitulated normal cord development in wildtype testis and the disrupted cord phenotype in null testes. TGFβ2-treated cultures, 13.5 dpc wildtype mouse testes displayed a 14-35% reduction in total area compared to untreated cultures. Null testes exhibited significantly smaller reductions in gonadal area (2-13%; $p<0.01$), indicating that betaglycan null testes exhibit reduced sensitivity to TGFβ2-mediated growth inhibition. However, preliminary observations suggest that TGFβ2 treatment partly rescued cord formation in two of three betaglycan knockout testes in vitro, with testis morphology confirmed by laminin and AMH immunostaining. These data support the notion that TGFβ2 acts via betaglycan to regulate cord development during foetal testis development. Supported by the New Investigator NHMRC (AUS) grant #550915 to MS, JKF Fellowship (#441101, #550915, #338516; #241000) and Victorian Government infrastructure funds.

(1) Sarraj et al., 2010. Biol Reprod; 82(1):153-62

169

THE MECHANISM OF SPERMATID MATURATION – A LINK TO TUMOUR SUPPRESSION

D. Jamsai[1,2], S. J. Smith[1,2], A. E. O'Connor[1,2], D. J. Merriner[1,2], C. Borg[1,2], B. Clark[1], V. Adams[3], C. J. Ormandy[4], M. K. O'Bryan[1,2]

[1]Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia
[2]The ARC Centre of Excellence in Biotechnology and Development, Monash University, Clayton, VIC, Australia
[3]The Australian Phenomics Facility, The Australian National University, Canberra, ACT, Australia
[4]The Garvan Institute of Medical Research, Sydney, NSW, Australia

To comprehensively uncover novel male fertility regulators, we utilised an unbiased forward genetic screen, ENU mutagenesis. Using this approach, we have identified several novel infertile mouse lines including a male-specific infertile line that we designated "Joey".

The mutant Joey mice produced no sperm due to an arrest of male germ cells at the round spermatid stage. The mutation was identified in the RNA binding motif 5 (*Rbm5*) gene that resulted in an arginine to proline substitution within a highly conserved RNA recognition motif of the protein. The substitution of proline is likely to interfere with RNA binding and/or recognition. In humans, the *RBM5* gene maps to a region that is frequently deleted in lung cancers. *Ex vivo* studies have suggested that RBM5 is a tumour suppressor, apoptosis modulator and RNA splicing regulator. To date, the role of *Rbm5* has never been liked to male fertility and the Joey line is the only mouse model of *Rbm5* dysfunction.

Using our RBM5-specific antibody, we showed that RBM5 is expressed in pachytene spermatocytes and round spermatids. Based on the protein localisation, the proposed role of RBM5 in mRNA processing, the onset of the Joey phenotype, and the site of the identified mutation, we hypothesise that the *Rbm5* mutant allele results in a hypomorphic protein, and that RBM5 has an essential role in regulating male germ cell mRNA storage, transport and/or translational regulation of mRNAs that are critical for spermatid maturation. Further, we generated mice compound heterozygous of the Joey *Rbm5* mutation and *Rbm5* null alleles. We showed that the compound heterozygous males are infertile due to spermatid maturation arrest resembling the Joey mutant males. This result further confirmed the identification of the *Rbm5* mutation as a cause of infertility in the Joey mice and a crucial role of *Rbm5* in male fertility.

THE ROLE OF MEGALIN IN PROSTATE DEVELOPMENT OF THE MOUSE

M. Gamat, G. Shaw, M. B. Renfree

Department of Zoology, The University of Melbourne, Parkville, VIC, Australia

Prostatic development is dependent on androgens; but the precise mechanism by which androgens mediate their effect is still unclear. Megalin, a cell membrane transporter, may shuttle sex steroids into cells to regulate androgen-responsive genes responsible for prostatic bud induction in the urogenital sinus (UGS). In megalin knockout mice, testicular descent fails and the vagina fails to open in females, both of which are dependent on sex steroid signalling (Hammes et al. 2005) . In this megalin-mediated pathway, SHBG-bound sex steroids bind to megalin, which is internalised. The SHBG-sex steroid complex is released, and the sex steroid is released from SHBG where it can bind to the androgen receptor to regulate androgen responsive genes. Receptor-Associated Protein (RAP) is a molecular chaperone protein that protects newly synthesised megalin from binding to potential ligands in the cytoplasm prior to insertion into the cell membrane. We hypothesised that megalin may shuttle SHBG-bound androgens across the cell membrane. This study characterised the expression and evaluated a possible role for megalin in the development of the mouse prostate.

Megalin, SHBG and RAP transcripts were detected in the developing male and female UGS of the mouse from day E14.5 to day E18.5 (when prostatic buds start to form) and in the adult prostate. Megalin, SHBG and RAP protein were localised in the urogenital epithelium. To assess the role of megalin in prostatic development, UGS tissues were incubated with androgens in the presence and absence of RAP. Incubating UGS tissues with RAP did NOT inhibit prostatic bud initiation. Furthermore, in the UGS of megalin knockout mice, prostatic bud formation appeared to be identical to those of wild-type littermates. These results demonstrate that megalin is not involved in prostatic bud initiation. However, the ubiquitous expression of megalin suggests that its role is redundant in the prostate.

(1) Hammes, A et al. (2005) Role of endocytosis in cellular uptake of sex steroids. Cell 122(5), 751-62

401

MECHANISMS OF ERB ACTION IN PROSTATE CANCER

R. Aesoey[1], E. F. Need[2], A. Trotta[2], G. Buchanan[2], G. P. Risbridger[1]

[1]Department of Anatomy and Developmental Biology, Monash University, Clayton, VIC, Australia
[2]Freemasons Foundation Centre For Men's Health, The University of Adelaide, Adelaide, SA, Australia

Androgen-deprivation remains the predominant effective therapy for advanced prostate cancer (PCa), but invariably fails with the emergence of fatal castrate-resistant disease. One potential new therapeutic option is the oestrogen receptor beta (ERβ) specific agonist (8beta-VE2), recently found to act via TNFα causing apoptosis in castrate-resistant cells. In contrast ERα is known to promote malignant prostate cell growth. Although there is 95% homology between the DNA-binding domains of the two isoforms, in breast cancer cell lines they exhibit both shared and selective gene-targets, and thus divergent transcriptional effects are expected. As little is known about ERβ in PCa our aim was to identify specific gene-targets with view to understand the determinants of PCa growth regulation. Expression of both TNFα and the "early" gene IL8 was induced by 8beta-VE2 treatment of human androgen-independent prostate DU145 cells indicating that these genes are targets of ERβ regulation in DU145 cells. ERβ activity on a consensus oestrogen-responsive-element (ERE) was induced by 8beta-VE2 treatment of DU145 cells. No activity of either ERα on the ERE or AR activity on the probasin reporter was induced by 8beta-VE2 in contrast to positive controls with oestradiol and DHT respectively. This suggests that the growth inhibition of DU145 cells by 8beta-VE2 is likely due to activation of ERβ and any promiscuous off-target effects through ERα or AR are unlikely. Only transfected ERα was detected in DU145 cells using an ERα specific antibody. However both endogenous and transfected ERβ were detected by an ERβ specific antibody , while transfected ERα was not, confirming antibody specificity. We are currently optimizing Chromatin Immunoprecipitation (ChIP) assays with ERβ and 8beta-VE2 in these cells with view to conduct genomic sequencing and matched expression microarrays. The identification of new ERβ gene-targets is essential to understand ERβ-agonist induced apoptosis that may ultimately improve therapy for advanced incurable PCa.

402

DEFINING THE ROLE OF ESTROGEN AND MAST CELLS IN PROSTATITIS AND THE DEVELOPMENT AND PROGRESSION OF PROSTATE CANCER

B. Cawsey, B. Niranjan, H. Wang, G. Risbridger, S. Ellem

Anatomy & Developmental Biology, Monash University, Clayton, VIC, Australia

In addition to androgens, estrogens also play an important role in the prostate and exert both adverse and beneficial effects, via estrogen receptors α (ERα) and β (ERβ), respectively. The adverse effects of estrogen are driven by ERα and include the pathogenesis of prostatitis and prostate cancer (PCa) (1). However, the specific factors and mechanisms mediating these effects remain unknown.

Mast cells are important mediators of inflammatory responses and have been implicated in a number of cancers, where they may be adverse or beneficial (2, 3). Although equivocal, mast cells have also been associated with poor prognosis in PCa and shown to respond directly to estrogen (4, 5). Thus, we hypothesized that mast cells may be important for mediating the development of inflammation and PCa in response to estrogen.

This concept was supported by our recent work examining the aromatase over-expressing mouse. This model demonstrated a progressive link between excess estrogen exposure, the development of prostatitis, and prostatic premalignancy. Significantly, mast cell numbers were increased prior to the development of inflammation, supporting their role in the initiation and progression of this continuum (6).

We have further examined the role of estrogen and its effect on mast cells using the human HMC-1 mast cell line. Initial results demonstrate ERα and ERβ expression in these cells, as well as increased proliferation in response to estrogen. Further experiments to define the inflammatory response to estrogen and the specific role of ERα in these cells are currently underway .

Ultimately, t his work will provide novel data defining the role of estrogen in the prostate, and specifically the role mast cells. This will lead to a better understanding of prostatic mast cell biology and increase our understanding of how estrogen exposure may be linked to the development of prostatitis and PCa.

(1) ELLEM et al. 2007. Nat Rev Cancer, 7, 621-7
(2) METZ, et al. 2007. Immunol Rev, 217, 304-28
(3) RIBATTI, et al. 2009. Int Rev Cell Mol Biol, 275, 89-131
(4) NONOMURA, et al. 2007. British Journal of Cancer 97, 952-956
(5) ZAITSU, et al. 2007. Mol Immunol, 44, 1977-85
(6) ELLEM, et al. 2004. J Clin Endocrinol Metab, 89, 2434-41

403

EXPRESSION AND CELLULAR ACTIVATION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA IN GRANULOSA CELL TUMOURS

S. Chu, Y. Chee, M. Alexiadis, P. J. Fuller

Steroid Receptor Biology, Prince Henry's Institute, Clayton, VIC, Australia

Granulosa cell tumours (GCT) of the ovary are rare, hormonally-active neoplasms characterised by endocrine manifestations, an indolent course, and late relapse. Chemotherapy and hormonal therapy have proved to be of limited efficacy. Nuclear receptors (NR) are well defined targets which have a central pathogenic role in endocrine malignancy. They are potential targets for therapeutic intervention. NR have established roles in granulosa cell biology but their roles in GCT remain largely unexplored. In order to more systematically examine the NR family in GCT, we used ABI Low Density Array microfluidic cards to analyse 14 GCT and two GCT-derived cell lines for expression of the 48 NR. The levels of expression were remarkably consistent across the GCT. We found that peroxisome proliferator-activated receptor gamma (PPARγ)) had greater than 10 fold absolute expression when compared with either the NCBI tumour or brain reference RNA pools. PPARγ) agonists are regarded as potential therapeutics in the treatment of inflammatory diseases and certain cancers. Given the high expression levels of PPARγ) in GCT, we investigated whether the use of PPARγ) and/or retinoid X receptor (RXR) agonists or antagonists have an effect on GCT-derived cell lines. We observed that the PPARγ) /RXR agonists and antagonists had no affect on cell proliferation, cell viability or apoptosis. Although the use of PPARγ) agonists is unlikely to be of use in treating GCT, a combination of therapies involving knockdown of NF-κB signalling may be of benefit. We have previously observed that several other members of the steroid receptor family are transrepressed due to constitutive activation of the NF-κB signalling pathway. We are currently investigating whether PPARγ) is transcriptionally active in these cells using a reporter construct specific for PPARγ) and whether the non-responsivness to PPARγ) agonists or antagonists in vitro is due to NF-κB transrepression.

404

NOVEL EFFECTS OF MELATONIN ON OESTROGEN PRODUCTION IN POST-MENOPAUSAL BREAST CANCER

K. C. Knower[1], H. Sasano[2], E. R. Simpson[1], C. D. Clyne[1]

[1]Cancer Drug Discovery Laboratory, Prince Henry's Institute, Clayton, VIC, Australia

[2]Department of Pathology, Tohoku University School of Medicine, Sendai, Japan

In contrast to therapeutic agents that may present adverse effects, the main biological active substance secreted by the pineal gland, melatonin (MLT), is known to counteract the effects of oestrogens in breast cancer (BC) via exerting a number of its own oncostatic properties. MLT is a hormone secreted into the bloodstream by the pineal gland in response to darkness, as such, levels rise and fall throughout the day leading to the hypothesis that an increased risk for BC exists among women with altered circulating MLT levels. Recent studies of post-menopausal women, has revealed that the major metabolite of MLT is statistically significantly associated with a lower risk of developing BC. While MLT production decreases with age; breast cancer risk, however, increases with age and obesity. In this study, we hypothesise that MLT inhibits oestrogen production in breast adipose tissue (the local source of oestrogen in post-menopausal women) by inhibiting transcription of the *CYP19A1* gene that encodes the key enzyme aromatase. Our keys findings show that (i) human breast adipose fibroblasts (BAFs) express the G-protein-coupled MLT receptors MT1 and MT2, as well as the nuclear hormone receptors of the RAR-related orphan receptor family; (ii) treatment of BAFs with physiological concentrations of MLT results in significant inhibition of CYP19A1 transcripts; and (iii) cancer-associated fibroblasts may prove a beneficial therapeutic target of MLT.

405

STROMAL HEDGEHOG SIGNALING MEDIATES PROSTATE EPITHELIAL TRANSFORMATION

S. Wilkinson[1], G. Buchanan[3], L. Martelotto[2], H. Wang[1], G. P. Risbridger[1], R. A. Taylor[1]

[1]Prostate & Breast Cancer Research Program, Monash University, Clayton, VIC, Australia

[2]Monash Institute of Medical Research, Monash University, Clayton, VIC, Australia

[3]The Freemasons Foundation Centre for Mens Health, University of Adelaide, Adelaide, SA, Australia

Background: The Hedgehog (Hh) signaling pathway is not only crucial for development, but has more recently been implicated in the initiation and promotion of a range of cancers [1]. Typically, Hh ligands are secreted by the epithelium, whilst receptors and downstream target genes are found in the stroma. It was recently demonstrated that in addition to canonical Hh target genes, there are a set of prostate-specific target genes regulated by Hh signaling involved in both development [2] and cancer [3]. Our overall aim is to determine whether stromal Hh signaling is responsible for malignant transformation of prostatic epithelial cells, either by canonical and/or prostate-specific Hh target gene regulation. *Methods & Results:* To determine if stromal Hh signaling contributes to carcinogenic transformation of prostatic epithelium, we analysed human prostate carcinoma-associated fibroblasts (CAFs) using genome wide Affymetrix arrays. Although canonical Hh target genes such as *Gli1* and *Hip1* were equally expressed in both CAFs and non-malignant patient matched fibroblasts (NPFs), we did observe significant upregulation of 6 prostate-specific Hh target genes; *Fgf5*, *Fbn2*, *Hes1*, *Hsd11β1*, *Igfbp6* and *Adamts12* in prostatic CAFs compared to NPF controls. *Discussion:* Since we and others have demonstrated that CAFs are capable of inducing carcinogenesis of prostatic epithelium, these results indicate that stromal Hh signaling may be a critical inducer of prostate cancer induction, potentially via non-canonical signaling pathways. Our ongoing studies aim to test the effect of constitutive stromal Hh signaling

by overexpressing Smo in human prostatic fibroblasts and functional tumourigenic testing in tissue recombination experiments. *Conclusion:* Stromal Hh signaling is active in human prostatic tumour stroma as seen by the upregulation of non-canonical Hh target gene expression. Further studies are required to determine whether stromal Hh signaling is capable of initiating prostate cancer in normal prostatic epithelium.

(1) Taylor, R.A., et al., Lineage enforcement by inductive mesenchyme on adult epithelial stem cells across developmental germ layers. Stem Cells, 2009. 27(12): p. 3032-42.
(2) Yu, M., et al., Sonic hedgehog-responsive genes in the fetal prostate. J Biol Chem, 2009. 284(9): p. 5620-9.
(3) Shaw, A., J. Gipp, and W. Bushman, The Sonic Hedgehog pathway stimulates prostate tumor growth by paracrine signaling and recapitulates embryonic gene expression in tumor myofibroblasts. Oncogene, 200

406

ENTRAPPING A BODY-BUILDER.A CASE REPORT

W. J. Braund

Endocrinology, Tennyson Centre, KURRALTA PARK, SA, Australia

This report describes a patient, with massive muscle hypertrophy associated with administration of anabolic steroids, who developed a bilateral anterior compartment syndrome with muscle ischaemia and nerve and artery compression. Emergency surgery was successful in preventing muscle necrosis of the anterior compartment. There is a handful of previous reports of this complication – which are briefly discussed. The "regimen" of medications supplied by his bodybuilding associates was complex and is presented in full: it includes synthetic androgens, HCG and tamoxifen.

(1) Liem NR, et al 2005 Acute exertional compartment syndrome in the setting of anabolic steroids: an unusual cause of bilateral footdrop. Muscle Nerve 32(1) 113-7
(2) Orava S, et al 1998 Chronic compartment syndrome of the quadriceps femoris muscle in athletes. Diagnosis and treatment with fasciotomy. Ann Chir Gynaecol 87 (1) 53 – 58
(3) Bahia H, et al 2000 Anabolic steroid accelerated multicompartment syndrome following trauma. Br J Sports Med 34 (4) 308-9
(4) Farkash U, et al 2009 Rhabdomyolysis of the deltoid muscle in a bodybuilder using anabolic-androgenic steroids: a case report. J Athl Train 44 (1) 98-100

407

TUMOUR SHRINKAGE AND IMPROVED HEADACHE WITH OCTREOTIDE IN A TSH AND GH CO-SECRETING PITUITARY MACROADENOMA

A. S. Cheung, P. Colman, S. Fourlanos

Department of Diabetes and Endocrinology, Royal Melbourne Hospital, Parkville, VIC, Australia

TSH secreting tumours(TSHoma) constitute 0.5% of all pituitary tumours. Of these, 30% co-secrete GH, prolactin or gonadotropins in order of decreasing prevalence1. In GH/TSH co-secreting tumours, thyrotoxicosis may be milder than expected and acromegalic features (present in 95%) may overshadow hyperthyroid features.

A 53-year-old previously well woman presented with 2 years of progressively worsening headaches. She had associated fatigue, postural dizziness and nausea. Four years prior, she had lost 25 kg in weight (body mass index 21) and her shoe size had increased by 1 size.

Examination features were consistent with acromegaly and there were signs of thyrotoxicosis with tachycardia and fine tremor of her hands. MRI pituitary confirmed a 2.2x2.5x1.8cm pituitary macroadenoma abutting the optic chiasm. Her growth hormone(GH) was 34.2mIU/L(<24) and IGF-1 67.3nmol/L(11.3-30.9). Thyroid function tests revealed TSH 1.16mIU/L(0.1-4.0) with elevated FT4 64.4pmol/L(9.0-26.0)and FT3 15.6 pmol/L(3.5-6.5). Alpha subunit was elevated at 0.57 IU/L(0.05-0.40). She had evidence of hypopituitarism with oestradiol 32pmol/L, FSH 2.5IU/L, LH 2.1IU/L, cortisol 113nmol/L, ACTH 2.5pmol/L(<20) and a raised prolactin 1019mIU/L(60-620).

Subcutaneous Octreotide therapy (100mcg twice daily) resulted in rapid normalisation of her TSH, fT4 and fT3 in 2 weeks with complete resolution of her headaches. Prolactin fell to 400mIU/L and pituitary MRI demonstrated a 40% reduction in tumour size at 3 months.

The patient was changed to Octreotide-LAR, and had an immediate return in severe headaches necessitating opiate analgesia. With return to short-acting Octreotide, her headaches once again abated. The analgesic effect of short-acting octreotide has also been observed in other patients2.

Surgery has traditionally been first line treatment, however, achieving euthyroidism preoperatively is paramount. Preoperative octreotide is effective in lowering TSH and GH levels and may have effects in reducing tumour size. With sustained reduction in tumour size, Octreotide may be a suitable first line treatment for patients with TSHoma.

(1) Beck-Peccoz P, Brucker-Davis F, Persani L et al. Thyrotropin-Secreting Pituitary Tumors. Endocrine Reviews 1996;17:610-638.
(2) Levy MJ, Matharu MS, Meeran K et al. The clinical characteristics of headache in patients with pituitary tumours. Brain 2005; 128, 1921–1930

408

THE ENIGMA OF ECTOPIC ACTH SYNDROME: CASE REPORT AND UPDATE

C. Lo[1], J. D. Zajac[2,3], E. Gianatti[2,3]

[1]currently at Department of Endocrinology and Diabetes, Southern Health, Clayton, VIC, Australia
[2]Department of Endocrinology, Austin Health, Heidelberg, VIC, Australia
[3]Department of Medicine, University of Melbourne, Heidelberg, VIC, Australia

A 29 year old man was referred with a 1 year history of migratory bony pains, facial rounding, fatigue, proximal sympathy, and decreased mood. A diagnosis of Melorheostosis, a rare congenital dystrophic bone disorder, had been made based on multiple sclerotic lesions in the axial skeleton found on CT and MRI scan. ACTH dependent Cushing Syndrome (CS) was confirmed by 2 elevated 24 hr urinary free cortisol readings of over 2800 nmol/day, failure to suppress on a low dose overnight dexamethasone suppression test (DST) and an elevated ACTH of 167.9 ng/L.

Further testing included:

Test	Result
8 mg overnight DST	Failure to suppress (Cortisol 481 nmol/L)
Pituitary MRI	Potential right sided 4 mm pituitary adenoma
Bilateral inferior petrosal sinus sampling	The central to peripheral ratio for ACTH was < 1.5 at baseline and < 3 after CRH stimulation.
CRH stimulation testing	Consistent with ectopic ACTH production

CT chest, abdomen and pelvis did not reveal a primary source. Urinary 5HIAA, serum calcitonin, glucagon, chromogranin A and serotonin were all within normal limits, while serum pancreatic polypeptide levels were marginally elevated at 62.7 pmol/L (NR 111In-pentetreotide scintigraphy but the lesions were PET scan avid. Bone marrow aspirate trephine of the left iliac spine revealed a moderately well differentiated metastatic carcinoid tumor staining positive for ACTH and calcitonin.

Antiadrenal medical therapy was commenced with ketoconazole but was poorly tolerated. Metyrapone, produced significant reductions in serum cortisol, 24 hour urine cortisol and resolution of the clinical syndrome. Introduction of lanreotide allowed a reduction in metyrapone dose without a change in the ACTH level. Currently, no evidence of tumor progression is evident with stable alkaline phosphatase and pancreatic polypeptide levels.

Ectopic ACTH Syndrome (EAS) accounts for 10% of cases of CS. As demonstrated in our case, common management issues posed by this syndrome include:

1) establishing the diagnosis of EAS

2) localisation of the primary tumor

3) management of the hormonal excess and tumor.

New and novel strategies for the management of EAS and carcinoid tumors include somatostatin analogues such as octreotide or lanreotide, radio-labelled somatostatin analogues, everolismus, and agents inhibiting the VEGF pathway.

409

MULTIFOCAL FIBROSCLEROSIS IN A PATIENT WITH A PROGRESSIVE GOITRE AND HYPOTHYROIDISM

C. M. Girgis, B. L. Champion

Endocrinology, Nepean Hospital, Kingswood, NSW, Australia

A 37-year-old female presented with a 5-month history of neck discomfort, low-grade fever and myalgia. She was clinically hypothyroid, with a diffusely enlarged, mildly tender thyroid gland and no palpable nodules or lymphadenopathy. Investigations confirmed hypothyroidism with strongly positive thyroid antibodies: TSH 110mU/L (reference range 0.4 – 4.7mU/L); Free T4 3.3pmol/L (reference range 9 – 28pmol/L); Free T3 of <1pmol/L (4.26 – 8.1pmol/L); T hyroglobulin antibody 592U/mL (0 – 60U/mL); Thyroid peroxidase antibody 2000U/mL (0 – 35U/mL).

Following a provisional diagnosis of Hashimoto's thyroiditis, increasing doses of levothyroxine failed to render the patient euthyroid. The goitre progressively enlarged with the development of upper airway obstructive symptoms. Constitutional symptoms became more prominent with accompanying leukocytosis, normochromic normocytic anaemia and elevated ESR of 90mm/Hr.

A CT scan of the neck demonstrated a large goitre with partial tracheal compression. An abdominal CT scan (to exclude occult malignancy) demonstrated a poorly defined pelvic lesion causing left hydronephrosis. A thyroid core biopsy demonstrated patchy lymphocytic infiltration and hyalinized fibrous stroma replacing thyroid parenchymal tissue, consistent with Riedel's thyroiditis. A later biopsy obtained during uretolysis, confirmed retroperitoneal fibrosis.

A diagnosis of multifocal fibrosclerosis was made. This is a rare fibro-inflammatory condition of uncertain aetiology, which encompasses Riedel's thyroiditis, retroperitoneal fibrosis, and a broad range of other fibrotic conditions. It is primarily an immune condition, which may be associated with thyroid autoantibodies and may respond to immunosuppressive agents.

There was marked reduction in the patient's goitre following high dose glucocorticoid therapy and ureteric stent insertion successfully relieved the hydronephrosis.

BONSOY AND IODINE: TOO MUCH OF A GOOD THING

K. Hughes[1], B. Crawford[1,3], E. Chua[1,3], C. Cowell[2,3]

[1]Royal Prince Alfred Hospital, NSW, Australia

[2]Institute of Endocrinology and Diabetes, The Children's Hospital at Westmead, NSW, Australia

[3]The University of Sydney, NSW, Australia

On Christmas Eve 2009, Food Standards Australia New Zealand (FSANZ) initiated a national recall of soymilk 'Bonsoy'. [1] The recall was the result of a cluster of eight adults with thyroid problems (Table 1) and one infant with neonatal hypothyroidism attributable to this soymilk. Testing of Bonsoy at Royal Prince Alfred, Sydney and NSW food-testing lab (DAL) revealed iodine content of 25,000-31,000 µg/L. Three other brands of soy milk tested had iodine contents of only 20 µg/L. The source of iodine in Bonsoy was Kombu ('sea-veg') a deep sea kelp added for flavour.

Table 1: Initial cluster of eight adult patients with thyroid problems attributed to Bonsoy consumption

Date presented	Sex	AGE	TSH (0.4-3.5)	fT4 (9-19)	fT3 (2.5-5.7)	Urinary iodine (ug/L)	TR Ab	TPO, TG Ab	thyroid US	Scan (0.5-3.5%)
20/11/08	F	36	4.63	9.7	n/a	4,445	nd	neg	nd	nd
1/02/09	M	38	<0.02	56	16	1,278	neg	neg	normal	<0.5%
2/12/09	F	37	<0.0005	29	5.6	6,208	neg	neg	normal	nd
12/11/09	F	46	<0.005	50	39	11,427	neg	neg	enlarged, reduced vascularity	<0.5%
19/11/09	F	36	<0.04	30	12	777	neg	neg	normal	0.5%
2/12/09	F	29	0.04	16	4.9	≠ceased Bonsoy prior to testing *48≠*	neg	neg	nodules <3mm	0.5%
10/12/09	F	33	0.08	18	6.6	5,022	neg	neg	normal	1.3%
10/12/09	M	47	0.07	17	4.9	320	neg	neg	0.5 cm nodule	0.1%

Mild iodine deficiency is common in Australia leading to mandatory iodine fortification of most bread since October 2009. [2] The RDI for iodine is 150 µ g for non-pregnant adults, with a recommended upper limit of 1,100 µ g. [3] Intake of just 35mL of Bonsoy a day would exceed the recommended daily safe upper limit of intake.3

The effect of iodine excess on the thyroid (which usually results from intravenous iodinated contrast, ingestion of seaweed, or drugs such as amiodarone) are well-known and can vary from hyperthyroidism to hypothyroidism. The reasons for this are unclear and may relate to the age of the patient, the presence of pre-existing autoimmune thyroid disease and the amount and duration of iodine ingested. [4] , [5] The adults presented here, however, did not appear to have underlying nodular goitres nor Hashimoto's. Iodine induced thyroid disease should be considered in cases of thyrotoxicosis at all ages with negative thyroid antibodies and low or absent uptake on a thyroid nuclear uptake scan.

[1] Weblink:http://www.foodstandards.gov.au/scienceandeducation/factsheets/factsheets2009/bonsoysoymilkrecall24652.cfm Accessed 8th June 2010

[2] Weblink: http://www.foodstandards.gov.au/consumerinformation/fortification/

[3] Nutrient Reference Values for Australia and New Zealand. Department of Health and Ageing. National Health and Medical Research Council. 2006. Available at http://www.nhmrc.gov.au Accessed 24th june 2010

[4] Markou K, Georgopoulos N, Kyriazopoulou V, Vagenakis AG. Iodine-induced hypothyroidism. Thyroid 2001; 11: 501-510.

[5] Roti E, Uberti ED. Iodine excess and hyperthyroidism. Thyroid 2001; 11: 493-497.

411

AN INTRIGUING CASE OF CUSHING'S

A. Joham

Department of Diabetes, Southern Health, Clayton, VIC, Australia

Clinical scenario

Mrs KS is a 53 year old woman living at home with her stepson, who was sent in to the Emergency Department by her general practitioner for poorly controlled diabetes. Insulin was added to her diabetes treatment regime at rapidly escalating doses. Patient compliance was questioned, but there was no glycaemic improvement even with supervised insulin administration. She had some clinical stigmata consistent with Cushing's syndrome. She denied any history of glucocorticoid therapy. Initial screening with a 24 hour salivary cortisol was elevated. Further extensive testing for Cushing's syndrome revealed perplexing results.

Investigations

· Midnight salivary cortisol: 23 (normal range < 9 nmol/L)

· Overnight (1mg) dexamethasone supression test

9am cortisol: 95 nmol/L

· 24 hour urinary free cortisol

Cortisol excretion: 4471 nmol/day (normal range 40 – 450)

· High dose dexamethasone suppression test

	Day 0	Day 1	Day 2	Day 3	Day 4	
ACTH	< 2	< 2	< 2	< 2	< 2	(< 10 pmol/L)
AM Cortisol	487	293	278	206	85	(nmol/L)
24 hour urine						
Urine volume		4229	5355	4356	4036	(mL)
Urine cortisol		905	811	1059	1632	(nmol/L)
Cortisol excretion		3827	4343	4613	6587	(60 – 400 nmol/day)

· CT abdomen/pelvis: no adrenal lesion identified

· MRI pituitary: pituitary gland normal in appearance

· Urine steroid profile - suppressed androgens, progestins and cortisol metabolites

· Chromatographic profile - unidentified peak in profile

· Mass spectrometry results – will supply

Questions

· Review the steps in evaluation of Cushing's syndrome

· Drug interference with the evaluation of tests in the diagnosis of Cushing's

· Explore assay specificity and assay cross-reactivity

· Role of mass spectrometry

412

THUNDERCLAP IN THE TOILET

I. Lee[1], I. Sim[2], P. Ebeling[1]

[1]Western Hospital, Footscray, VIC, Australia

[2]St. Vincent's Hospital, Fitzroy, VIC, Australia

46 yo male presented to Emergency Department with sudden onset of a severe headache post-micturition lasting 10-15 mins associated with tremors, facial pallor, palpitations and a systolic blood pressure of 210. This was on a background of 18 months of post-micturition tremors and palpitations, which he attributed to stress at work , with episodic diarrhoea. These symptoms had progressively worsened in the week prior to presentation.

His past medical history included malaena in 2006 when he was investigated with a gastroscopy which showed an antral submucosal lesion, which could be a pancreatic rest, carcinoid or GIST. In 2009, he had also had a general anaesthetic for open reduction of a fractured right finger that was uncomplicated.

There was a high suspicion that his signs and symptoms were suggestive of a probable perivesical paraglanglioma. He was started on phenoxybenzamine. Investigations with 24hr urinary catecholamines and plasma metanephrines were significantly elevated confirming the diagnosis. A CT abdomen & pelvis showed a left lateral bladder lesion 35 x 32 x 32mm with uptake seen on a I-123 MIBG whole body scan.

On confirmation of a paraganglioma, patient was referred to Urology and a partical cystectomy completely excised the lesion (histology: characteristic of a paraganglioma) that was confined to the detrusor wall. Post-operatively, he remained normotensive and his catecholamines normalized. Due to the previous history of a possible GIST and paraganglioma, a CT chest was performed to look for a benign cartilaginous tumour of the bronchus associated with Carney's triad. This showed a tiny calcified nodule within the right upper lobe, possibly a pulmonary chondroma. The linkage between a paraganglioma, possible GIST and paraganglioma raised the suspicion of a Carney's triad.

Genetic testing undertaken showed a negative gene analysis for SDHB, SDHD, RET and VHL.

(1) Young W, Paraganglioma – Clinical Overview; Ann NY Acad Sci (2006), 1073: 21-29
(2) Siatelis A et al, Phaeochromocytoma of the urinary bladder: report of 2 cases and review of literature; Minerva Urol Nefrol, (2008), 60(2): 137-40
(3) Stratakis CA & Carney JA, The triad of paragangliomas, gastric stromal tumours and pulmonary chondromas (Carney triad), and the dyad of paragangliomas and gastric stromal sarcomas (Carney-Stratakis sy
(4) Neumann H, Distinct clinical features of paraganglioma syndromes associated with SDHB and SDHD gene mutations; JAMA (2004), 292: 943-51

413

A CASE OF SARCOIDOSIS WITH HYPERCALCEMIA AND RENAL INVOLVEMENT IN AN INDIGENOUS LADY

N. Nandi, S. Chitturi, D. Howard

Department of Endocrinology, Royal Darwin Hospital, Tiwi, NT, Australia

Literature on Sarcoidosis in indigenous population is scarce. In general, renal involvement in Sarcoidosis is an occasional problem. Renal involvement in sarcoidosis manifests commonly as nephrolithiasis , rarely as renal insufficiency. Hypercalcemia is less common than hypercalciuria. We present a case of Sarcoidosis with hypercalcemia, renal insufficiency and nephrolithiasis without hypercalciuria in an indigenous lady.

Our patient is a 63 y old Australian Aboriginal lady who presented with symptomatic hypercalcemia (corrected calcium 3.47 mol/l) and acute-on-chronic renal failure (Creatinine 190 umol/). Her physical examination was normal. She had suppressed PTH, normal 25(OH) D and elevated 1, 25(OH) D (346 pmol/L) and raised ESR. LFT, FBC, PTHrP and ACE were normal. Malignancy screen & Tuberculosis work up were negative. CT chest showed bronchiectasis in a small segment of right middle lobe, minor consolidation but no hilar lymphadenopathy. Spirometry showed restrictive pattern. Abdominal ultra sound & CT scan showed multiple renal parenchymal and calyceal stones but no ureteric stone. She was initially treated with Intravenous fluid and Pamidronate with symptomatic and biochemical improvement. She was discharged with regular follow up arrangements.

Four months later, she represented with the same problem. This time, CT chest showed presence of enlarged left axillary lymph nodes, biopsy of which showed non-caseating granulomatous lymphadenitis consistent with Sarcoidosis. Her 24 hour urinary Calcium, phosphate, oxalate were normal. After initial improvement of calcium and creatinine following treatment with intravenous fluid and Pamidronate, Prednisone 50 mg started after infectious disease screen. Subsequently calcium(2.25 mmol/l) and renal function (Creatinine is 125 mmol/l) improved significantly allowing prednisone to be weaned. She had a relapse because of poor adherence to prednisone.

(1) Michael C. Iannuzzi, et al 'Sarcoidosis' N Engl J Med 2007;357:2153-65.
(2) Berliner AR, et al. 'Sarcoidosis: the nephrologist's perspective' Am J Kidney Dis 2006;48:856-70

414

AYPICAL PRESENTATIONS OF PITUITARY APOPLEXY

R. Narayanan, B. Crawford, K. Benson

Endocrinology, Concord Repatriation General Hospital, Sydney, NSW, Australia

Pituitary apoplexy classically presents with sudden headache , nausea, vomiting, visual symptoms and altered mental state. Atypical presentations can confound diagnosis and delay prompt treatment. Three atypical presentations are described .

1) A 64 year old man presented 2 weeks after CABG with tiredness, nausea and poor appetite. He had postural hypotension but no visual field deficits or ophthalmoplegia. He was hyponatraemic (sodium 119mmol/L), hypocortisolaemic (0700 cortisol 36nmol/l) and hypothyroid (fT4 7.9pmol/L) with low TSH, LH, FSH, ACTH, GH, IGF-1 and prolactin. Brain MRI showed recent haematoma in the pituitary gland, superior bulging of the sella turcica and deviation of the pituitary stalk to the left.

2) A 33 year old pharmacist presented with awareness of a visual field defect, confirmed by formal testing to be an upper bitemporal quadrantanopia. Brain MRI showed a 2.6cm pituitary macroadenoma with cystic suprasellar extension and an area of recent haemorrhage. Mass effect was marked on the optic chiasm and mild on the hypothalamus. Pituitary hormone function was normal. There had been an episode of nausea 2 months prior for which she had sought medical review and had been prescribed pantoprazole; in retrospect, she had also experienced mild headache. These were the only symptoms that could have been attributed to recent pituitary haemorrhage.

3) A 16 year old male of Islander background presented for obesity management (wt 145 kg) and recent diagnosis of type 2 diabetes. His history included recent exacerbation of chronic headaches, now worse in the morning and associated with photophobia. A brain CT showed a 2.5cm mass arising from the pituitary gland and extending into the suprasellar region with optic nerve displacement. The lesion demonstrated ring enhancement and a fluid level; subsequent MRI features were consistent with recent haemorrhage. Biochemistry showed hyperprolactinemia (7030mIU/L: NR 40-450), low testosterone (1.9nmol/L) but normal TFTs, cortisol and IGF-1.

These cases highlight subtle presentations of pituitary apoplexy including hyponatremia, isolated nausea and relatively mild headache.

415

REVISITING THE DIAGNOSIS OF PHAEOCHROMOCYTOMAS

R. Narayanan, K. Benson

Endocrinology, CRGH, Sydney, NSW, Australia

The low prevalence of phaeochromocytomas can impede their clinical and biochemical diagnosis. Biochemical diagnosis is made with the demonstration of excess catecholamine production. Phaeochromocytomas can present with isolated elevation of either catecholamines or metanephrines and one such case is discussed here. Their biochemical and clinical phenotypes depend on the underlying genetic aetiology and different phenotypes are proposed for MEN2 and VHL.

A 70 year old lady was referred for further assessment of refractory hypertension whilst on multiple agents including spironolactone, olmesartan, diltiazem, metoprolol and moxonidine. She had a 30 year history of hypertension that had become difficult to control over the last 5 years. Her past history included primary hyperparathyroidism and multinodular goitre for which parathyroidectomy and thyroidectomy had previously been performed. She had noted increased generalised sweating without paroxysms of palpitations or headaches. She was obese (Wt 115.8kg, BMI 40 kg /m^2) and hypertensive (BP 160/90mmHg) without other features of hypercortisolaemia.

Investigations showed a low serum potassium (3.2mM), normal creatinine, low normal urinary potassium and low aldosterone to renin ratio whilst on an angiotensin receptor blocker, normal dexamethasone suppression test and urinary free cortisol. A spiral CT angiogram of the renal arteries excluded renal artery stenoses but showed 1.5cm left and 3cm right adrenal lesions with noncontrast attenuation figures of 4 and 40 Hounsfield units respectively. A 24 hour urine collection showed normal adrenaline and noradrenaline with elevated metanephrine (3.1umol/d, N < 5.1) and normetanephrine (11.6umol/d, N < 2.1). This was confirmed with elevated plasma metanephrine (2.65nmol/d, N < 0.4) and normetanephrine (1.2nmol/d, N< 0.9). ^{123}I-MIBG showed uptake in the right adrenal gland, no uptake in the left adrenal gland and no extra-adrenal disease.

The optimal diagnostic test for biochemical diagnosis and the utility of urinary versus plasma measurements are important considerations given that some cases are catecholamine negative but metanephrine positive. Effective screening should therefore incorporate both catecholamines and metanephrines.

416

ANOTHER REASON TO AVOID THE DENTIST: METASTATIC INSULINOMA

M. Ng Tang Fui[1], M. Grossmann[1,2]

[1]Endocrinology, Austin Health, Heidelberg, VIC, Australia

[2]University of Melbourne, Melbourne, VIC, Australia

A 48 year old Cambodian woman presented to her GP in 2007 with symptoms of gastro-oesophageal reflux. Liver ultrasound revealed 4 suspicious liver lesions up to 1.4cm in diameter. Fasting glucose in 2007 was 5.7mmol/L.

She attended her local tertiary hospital and further workup in early 2008 confirmed multiple liver lesions, a 20mm pancreatic head lesion and a 7mm nodule at the superior mesenteric vein on CT scan. Hormonal panel included glucose, glucagon, urine 5-HIAA, serum serotonin, urine catecholamines, serum calcium, U+E, FBE and were all normal. Chromogranin A was elevated at 21U/L (N: 0-17.2U/L). Insulin and c-peptide were not performed initially. Somatostatin scintigraphy did not reveal any increased uptake. Liver histology was consistent with metastatic neuroendocrine tumour with positive staining for chromogranin A and weak staining for enolase and synaptophysin.

The patient was referred to our liver transplant unit for consideration of Whipple's procedure with liver transplant but this was deemed not inappropriate. Despite two different chemotherapy regimens, the disease progressed radiologically and chromogranin A rose to 1,600U/L.

The endocrine Unit became involved when the patient presented in January 2010 with morning dizziness and tremor which improved after breakfast. BSL was 2.2mmol/L, insulin 15 (2.5-11mIU/L) and c-peptide 1.17 (0.33-1.47nmol/L). This was consistent with insulinoma. Repeat somatostatin scintigraphy revealed uptake in a supraclavicular lymph node. Initial therapy with diazoxide, prednisolone, transhepatic arterial chemoembolisation and regular meals stabilised her BSL.

Hypoglycaemia subsequently recurred 1 week after a visit to the dentist, resulting in tooth ache and an inability to maintain her oral intake. After stabilisation with analgesia, IV dextrose and hyperglycaemic therapy, everolimus, a mTOR inhibitor was commenced. The patient remains relatively well with BSL maintained above 6 despite extensive visceral, lymph node and bony metastases. No further dental work is anticipated.

Issues for discussion:

1. Tumour biology of metastatic insulinomas which differentiate following diagnosis
2. Techniques to assess for tumour metastasis
3. Role of surgery in metastatic disease
4. Modalities for biochemical control
5. Modalities for control of tumour burden

(1) Yao JC et al. Efficacy of RAD001 (Everolimus) and Octreotide LAR in Advanced Low- to Intermediate-Grade Neuroendocrine Tumors: Results of a Phase II Study. J Clin Oncol 2008;26:4311-4318

(2) Vezzosi D et al. Short- and long-term somatostatin analogue treatment in patients with hypoglycaemia related to endogenous hyperinsulinism. Clin Endocrinology 2008;68:904-911

(3) Gurusamy KS et al. Liver resection versus other treatments for neuroendocrine tumours in patients with resectable liver metastases. Cochrane Database of Systematic Reviews 2009, Issue 2. Art. No.: CD0

417

TO PRESENT A RARE CASE OF PSEUDO HYPOPARATHYROIDISM PRESENTING WITH POLYGLANDULAR FAILURE

S. Nilajgi, U. Malabu

Endocrinology, Townsville Hospital, Townsville, QLD, Australia

A 32 year old was diagnosed to have pseudo hypoparathyroidism type 1a (PHP-1a) based on clinical-biochemical features at age 11.Clinically she had a notable rounded face and short neck with short right 3rd, 4th and 5th and left 4th and 5th fingers and potter's thumb consistent with Albright's hereditary osteodystrophy (AHO). Biochemically she had hypocalcemia, hyperphosphatemia, elevated PTH with primary hypothyroidism, Addison's disease (AD) and growth Hormone (GH) deficiency.

Relevant investigations:

Test	Result	Normal range
Calcium corrected	Low 1.86	(2.2-2.55 mmol/L),
Phosphate	High 2.45	(0.6-1.55 mmol/L)
Parathyroid hormone (PTH)	High 7.9	(1.6-6.9 pmol/L)
Urinary calcium	< 0.5	(2.5 – 7.5 mmol) / 24 hours
Potassium	High 5.8	(3.2-4.5 mmol/L)
Glucose	Low 1	(3- 6 mmol/L)
Basal cortisol	Low 34	(200 – 700 nmol/L)
Synacthen test	Inadequate response 53	(>550nmol/L)
ACTH	High 1850	(10-50 ng/L).
Thyroid stimulating hormone (TSH)	High 30.46	(0.36-3.25 mU/L)
Free T4	Low 6.6	(9.4-25 pmol/L).
Growth hormone pre and post clonidine stimulation test	Low < 0.5	Post stimulation> 10mu/L

X-ray hands revealed shortening of 4th and 5th metacarpals on left and 3rd, 4th, 5th metacarpals on the right .

CT Brain showed Bi-basal ganglia calcification with asymmetry of lateral ventricles.

MRI Brain revealed normal pituitary gland.

Low basal cortisol level 34 nmol/L, inadequate synacthen response, elevated ACTH with hyperkalemia and hypoglycaemia confirmed Addison's disease. She is maintained on variable dosages of prednisolone and 100 ug of fludrocortisone for the AD.

She had primary hypothyroidism and remained euthyroid on L-thyroxine 100 ug once daily.

GH deficiency was confirmed on clonidine stimulation test.

Discussion: PHP-1a is a genetic disease characterised by clinical hypoparathyroidism caused by (PTH) resistance[1]. It consists of AHO and clinical hypoparathyroidism as in our case whereas type 1b has only biochemical features[2]. Our case is unique in having associated polyglandular failure namely AD, GH deficiency and primary hypothyroidism.

(1) 1 Tamanda Y, Kanda S, Suzuki H , Tajima T , Nishiyama t.A pseudohypoparathyroidism type 1a patient with normocalcemia. Endocr J 2008;
(2) 2 Levine, M. A. Pseudohypoparathyroidism: from bedside to bench and back. J. Bone Miner. Res 1999; 14: 1255-1260.

418

THERAPEUTIC OPTIONS IN TREATMENT-REFRACTORY ACROMEGALY

M. Pace, D. Topliss

Department of Endocrinology & Diabetes, The Alfred, Melbourne, VIC, Australia

Case 1

37-year-old man with florid acromegaly on a background of 5-10 years of increasing shoe and helmet size, bilateral carpal tunnel syndrome, skin tags, neck pigmentation and back pain. 6-12 months prior to presentation he developed headache and arthralgias.

He had gross changes of acromegaly involving the face, hands and feet, acanthosis nigricans and skin tags around the neck. Visual fields were intact to confrontation. Serum IGF-1 and GH were raised at 126nmol/L and 177mU/L respectively, with otherwise normal pituitary function. MRI pituitary showed a macroadenoma with no evidence of optic chiasmal or hypothalamic compression.

The patient was commenced on somatostatin analogue therapy and underwent transphenoidal tumour resection, followed by stereotactic radiotherapy and the addition of cabergoline. IGF-1 and GH improved but failed to normalise and he remained symptomatic. Pegvisomant 10mg/day was commenced in addition to somatostatin analogue and dopamine agonist therapy. There was no clear further improvement in IGF-1 over 6 months (53.3nmol/L), therefore the pegvisomant dose was increased to 20mg/day.

Case 2

41-year-old woman with a 13-year history of acromegaly associated with McCune-Albright syndrome (MAS) diagnosed at age 17 years. She had craniofacial fibrous dysplasia, skin pigmentation with café-au-lait spots and endocrine dysfunction of precocious puberty, hyperprolactinaemia and intermittent galactorrhoea, hirsutism and acromegaly.

A tall woman with broad sweaty hands, she had obvious facial asymmetry due to overgrowth of the right jaw and maxilla, and right temporo-parietal skull enlargement. There were no visual field defects, skin tags or acanthosis nigricans. Serum IGF-1 was elevated at 4.2U/mL [0.34-1.42] and GH at 12.7mcg/L, with a prolactin of 959mU/L. MRI pituitary showed a microadenoma and diffuse bony

expansion with heterogeneous enhancement of the right petrous apex, maxilla and base of skull including the pituitary sella. A zoledronic acid dose for control of bony growth caused transient right CN VI palsy.

Fibrous dysplasia of the skull precluded pituitary surgery and radiotherapy was considered contraindicated. She was treated with octreotide LAR complicated by alopecia, then lanreotide Autogel. IGF-1 remains elevated at 57.5nmol/L with a GH of 7.4mU/L. She is to commence pegvisomant therapy.

Clinical Questions

- What therapies are available in acromegaly refractory to surgical treatment and where treatment options are limited?
- What is the role of radiotherapy in acromegaly?
- What long-term safety and efficacy data is available for pegvisomant?

419

EXERCISE ASSOCIATED HYPONATRAEMIA: AN AUSTRALASIAN CASE SERIES

D. Pattison[1], E. Seal[2], T. Walters[2]

[1]Endocrinology, Royal Melbourne Hospital, Parkville, VIC, Australia
[2]Medicine, Royal Melbourne Hospital, Parkville, VIC, Australia

Background: Exercise-associated hyponatraemia (EAH) is a common, life-threatening illness due to conditioned overhydration defined as hyponatraemia occurring during or up to 24 hours after prolonged exercise [1] . Despite being well documented in scientific literature those most at risk (military personnel [2] , hikers [3] and endurance sports participants [4]) are often unaware of this preventable condition.

Cases: We present five cases of severe EAH. The first is a 43yo hiker on Kokoda Track in Papua New Guinea who developed seizures and was fortunately evacuated to an American naval hospital ship, intubated and treated with hypertonic saline (Na 114). The second case is a 21yo male New Zealand Defence Force soldier on deployment to Solomon Islands who presented with symptomatic EAH (Na116) and was identified to have a mutation associated with constitutive activation of X-linked AVPR2 (arginine vasopressin receptor type 2) consistent with the diagnosis of nephrogenic syndrome of inappropriate antidiuresis. The remaining cases include an Australian soldier in Solomon Islands and endurance cyclists in northern Victoria.

Discussion: Symptoms of EAH are similar to dehydration and commonly exacerbated by misguided attempts at rehydration [5] . The unexplained deaths of four young hikers on the Kokoda Track in 2009 associated with a culture of conditioned overhydration in similar conditions to these cases suggest EAH, not dehydration, was a more likely cause. Aetiology of EAH includes behavioural precipitants (excessive drinking, weight gain during exercise, NSAID use) and possible undefined underlying biological defects in free water excretion [1]. Case 2 is the first description of EAH associated with an activating mutation of AVPR2. Prevention of EAH includes limiting drinking to thirst and avoiding weight gain during prolonged exercise. Public health response should include education of endurance sports participants and doctors and revision of Australasian military heat illness prevention guidelines. Novel activating mutations of AVPR2 should also be considered in other clinical scenarios including SIADH with undetectable ADH levels [6] and hyponatraemia post pituitary surgery.

(1) Hew-Butler TD, Ayus JC, Kipps C, et al. Statement of the Second International Exercise-Associated Hyponatraemia Consensus Development Conference, New Zealand 2007. Clin J Sport Med. 2008; 18: 111-121.

(2) O'Brien KK, Montain SJ, Corr WP, et al. Hyponatraemia associated with Overhydration in US Army Trainees. Military Medicine, 2001; 166, 5: 405-410

(3) Rothwell SP, Rosengen DJ. Severe Exercise-Associated Hyponatraemia on the Kokoda Trail, Papua New Guinea. Wilderness and Environmental Medicine, 2008; 19: 42-44.

(4) Almond CSD, Shin AY, Fortescue EB, et al. Hyponatraemia among runners in the Boston Marathon. N Engl J Med 2005; 352:1550-1556.

(5) Gardner JW. Death by water intoxication. Military Medicine 2002; 167, 5: 432-434.

(6) Speedy DB, Noakes TD, Rogers IR et al. A prospective study of exercise-associated hyponatraemia in two ultradistance triathletes. Clin J Sport Med 2000; 10: 136-141.

420

THE COMPLEXITY OF LABORATORY TESTING AND DIAGNOSIS OF STEROID EXCESS SYNDROMES ASSOCIATED WITH HERBAL REMEDY USE.

N. Perera[1,2], B. Crawford[1,3], E. Chua[1,3]

[1]Endocrinology, Royal Prince Alfred Hospital, Sydney, NSW, Australia
[2]Clinical Biochemistry, Royal Prince Alfred Hospital, Sydney, NSW, Australia
[3]Sydney Medical School, University of Sydney, Sydney, NSW, Australia

Context: Global demand for and use of 'natural' or herbal medicines in this era of extensive travel and internet trade has increased. Surveys have shown that majority of western populations seek non-drug solutions via complementary medicines and spend billions of dollars on them each year. The unreliability of these products with risk of contamination with undeclared synthetic pharmaceuticals are less recognised and under reported.

Objective: The objective is to highlight some of the harmful consequences of such adulterants in these seemingly harmless "natural" preparations and to draw attention to the complexity and increased vigilance needed when interpreting clinical presentations and routine biochemical tests.

Design and measurements: This report describes a patient presenting with clinical features of steroid excess but has unmeasurable serum cortisol, suppressed ACTH and low 'normal' urine free cortisol(UFC) when tested on routine immunoassays.

Results: Gradual withdrawal of the herbal supplements used by the patient resulted in regression of presenting features and normalisation of hypothalamic-pituitary-adrenocortical(HPA) axis.

Conclusions: This case highlights the complexity of interpreting routine pathology including an unusual situation of false negative cortisol results with herbal remedy use. It also highlights the great need for clinicians to appreciate the robustness (or otherwise) of their particular assays as well as the need for assessment of safety, efficacy and quality control of 'herbal' products.

421

MYOSITIS ASSOCIATED WITH TREATED HYPERTHYROIDISM

A. N. Pham, S. Perampalam, J. D. Wilson

Endocrinology, Canberra Hospital, Canberra, ACT, Australia

Context: Musculoskeletal complaints are a well-known association with thyroid disease and antithyroid drugs, however, myositis associated with treated hyperthyroidism is an uncommonly reported entity.

Case Report: A 23 year old female presented with a 2 month history of palpitations, alopecia and heat intolerance. There were no features of myalgias or myopathy. Her TSH was <0.05 mIU/L, free T4 52pmol/L and free T3 22.7pmol/L. She was diagnosed with Graves Disease and commenced Carbimazole 15mg tds.

One month following treatment initiation, she developed generalised myalgias which gradually worsened, resulting in hospital presentation (three months after her diagnosis) with severe cramps. Her total daily carbimazole dose was 25mg. She was not on any other medications. On examination, she had diffuse muscle tenderness with normal power and reflexes. Clinically she was euthyroid and there was no evidence of a connective tissue disease.

Her creatine kinase (CK) was elevated, 1440 U/L (RR 20-200). TSH 0.21 mIU/L (RR 0.4-4.0), free T4 9.7 pmol/L (RR 9-20) and free T3 4.6pmol/L (RR 2.6-5.7). Her ANA titre was 1:320 and the remainder of her autoimmune screen, biochemistry and Vitamin D were normal.

Carbimazole was ceased and her CK gradually returned to normal. Despite decline of CK her muscle pain did not improve. Following administration of Naprosyn and short term prednisolone, her symptoms improved. She was later commenced on propylthiouracil.

Discussion: We found 9 cases of myositis associated with treated hyperthyroidism in children and adults. The true prevalence of this condition is unknown as CK is not often evaluated despite muscular symptoms. The pathophysiology is unclear but muscle damage could be directly related to antithyroid drugs or secondary to relative hypothyroidism. A number of predisposing factors have been proposed. Recognition of this underreported condition may lead to slower correction of hyperthyroidism or substitution with another antithyroid drug.

422

INVESTIGATIONS AND MANAGEMENT CHALLENGES OF DEDIFFERENTIATION FOLLICULAR THYROID CANCER

A. Piotrowicz, R. Smith

Endocrinology, John Hunter Hospital, Newcastle, NSW, Australia

A 68 year old man with Graves Disease required three doses of therapeutic iodine 3.7, 6.48 and 3.8GBQ. Follow-up examination of his neck revealed a large right sided thyroid mass. Since FNA was suspicious for malignancy, total thyroidectomy with lymph node dissection was performed.The pathology confirmed locally invasive follicular carcinoma with large vessel invasion but no lymph node metastasis.Despite three doses of radioactive iodine doses his stimulated thyroglobulin levels continued to rise. Initially positive, the third post radioactive iodine whole body scan ceased to demonstrate uptake in the neck.However a PET scan showed a small focus of low grade uptake in the area previously evident on the whole body study.A neck thyroid ultrasound revealed multiple small nodules not visible on PET scan.

Discussion: Literature review of thyroid malignancy risk after radioactive iodine for Graves Disease and its impact on type of cancer detected and its severity

Iodine avid thyroid carcinoma-mechanism of dedifferentiation

Role of FDG PET in differentiated thyroid cancer with elevated thyroglobulin and negative 131I scan

- correlation of thyroglobulin with PET- false negative scan
- PET imaging under TSH stimulation/rhTSH may increase its sensitivity

Effectiveness of thyroid remnant ablation in differentiated thyroid cancer with low vs high doses of radioactive iodine Role of radiotherapy, chemotherapy and VGEF inhibitors

423

ACUTE ADRENAL INFARCTION: HOW OFTEN IS IT OVERLOOKED?

W. E. Plehwe

The Epworth Centre, Richmond, VIC, Australia

Five patients (3M, 2F) aged 59-85yr (mean 69.9yr) with bilateral adrenal infarcts resulting in adrenal failure were diagnosed during a 13 yr interval. Three (2M, 1F) had undergone routine primary total hip replacement (THR) for osteoarthritis, one (F) was known to have had anti-phospholipid syndrome (APL) – warfarin had been ceased prior to diagnostic pleurocentesis for pleural effusion, and one (M) had severe thrombocytopaenia due to SLE and had successfully undergone lumbar laminectomy 6m earlier. Of the patients undergoing THR, 2 were diagnosed during the same admission with presenting features of 1) epigastric pain, postural hypotension and fever, and 2) hyponatraemia and hypotension. The 3rd patient with THR had after discharge, presented repeatedly to a suburban hospital with hypotension and fever, and was finally diagnosed about 6m post-operatively. The patient (F, 59y) with APL presented with hypotensive collapse and hypoglycaemia (BGL 2.4 mmol/L). The final patient (M, 77y) with SLE, presented with a 3m history of anorexia, nausea, vomiting, malaise and weight loss (10kg). He had noted increased pigmentation and Na+ was 120 mmol/L. All responded to appropriate hormone replacement, however the patient with APL suffered catastrophic deterioration with extensive visceral infarction and did not survive. Primary Addison's disease and malignancy were excluded. Acute imaging demonstrated enlarged haemorrhagic adrenal glands in 2 cases post-THR, with FNAB revealing fibrin and necrotic debris only. Follow-up imaging showed atrophic adrenal glands consistently. Hormone and antibody data were consistent with past and current diagnoses. Na+ 120-136 mmol/L (mean 126), Cortisol 15-81nmol/L (45) and ACTH 127-259pmol/L (180). The patient with APL had anti-cardiolipin antibodies (IgG>100) and a lupus anticoagulant. The patient with SLE had positive ANA (>1:1280) and dsDNA antibodies [104 (Farr) and 18 (FEIA)]. Anti-adrenal antibodies were not detected. The 3 THR patients were previously well and this complication could not be anticipated. HITTS was not detected, but the cause is considered to be venous thrombosis. Case 3 demonstrated the rare complication of "catastrophic APL" considered to be arterial rather than venous thrombosis. Thrombocytopaenia in SLE is uncommon, but with HITTS is recognised as predisposing to acute adrenal haemorrhage with venous thrombosis. In summary, while rare, these cases delineate a potentially fatal in-hospital complication and highlight the need to consider adrenal failure in atypical presentations. In these patients, low serum cortisol was the only immediate abnormality, hyponatraemia developing subacutely.

424

CASE REPORT: ISOLATED ACTH DEFICIENCY PRESENTING AS SEVERE HYPERCALCAEMIA WITH ACUTE RENAL FAILURE.

P. Shanmugalingam, K. Benson

Department of Endocrinology & Metabolism, Concord Repatriation General Hospital, Concord, NSW, Australia

Whilst hypercalcaemia is well described in Addison's disease, it is not a recognized feature of hypoadrenalism due to pituitary failure. We describe a case of a 55 year-old man who presented with a four-week history of nausea, vomiting, lower abdominal pain and 8 kg weight loss. Initial blood tests showed markedly elevated corrected calcium of 4.32 mmol/L with a suppressed PTH level of 1.3 pmol/L (1.6-6.9). The patient was also in acute renal failure with a urea of 14.2 mmol/L and a creatinine of 448 umol/L. He was euthyroid, with a low 1,25 dihydroxyvitamin D level of 16.6 pmol/L (38-162). Myeloma screen, including bone marrow biopsy and skeletal survey were negative. Investigations for malignancy including PTHrP and imaging with CT scans, whole body bone and PET scans were all unremarkable.

The patient was treated with aggressive saline rehydration, intravenous zolendronic acid and high dose steroids. He responded very well, with normalization of renal function and calcium levels over the next ten days. Steroids were weaned off after two weeks. However, due to the elusive aetiology of the severe hypercalcaemia, morning serum cortisol and ACTH levels were ultimately measured (seven-weeks after cessation of steroids), and demonstrated low readings of 92 nmol/L and 2.0 pmol/L (0-12) respectively. A subsequent short synacthen test was suggestive of adrenal insufficiency with a baseline cortisol of 80 nmol/L and a 60-minute concentration of 433 nmol/L. Insulin tolerance test confirmed HPA axis suppression with a subnormal peak cortisol level of 454 nmol/L (>550). Importantly, the remainder of the patient's pituitary hormone profile was unremarkable.

A diagnosis of isolated ACTH deficiency was made, and the patient was commenced on prednisolone 5 mg daily. Although the initial pituitary MRI did not reveal any pathology, repeat imaging six-months later demonstrated a slightly prominent pituitary stalk with a relative lack of T1 hyperintensity on the neurohypophysis. The appearance was thought to be possibly in keeping with lymphocytic hypophysitis. Notably, institution of glucocorticoid therapy resulted in a marked improvement in the patient's clinical state, and on follow-up at 18 months, he remains normocalcaemic.

425

AN INTERESTING CASE OF LYMPHOCYTIC HYPOPHYSITIS AND THYROIDITIS

E. N. Suthaharan, A. Sinha, J. Yarker, Y. Raje, A. McLean

Endocrinology and diabetes, Cairns Base hospital, Cairns, QLD, Australia

This is a case of a lady with a pituitary mass in pregnancy, who later developed thyrotoxicosis, pituitary insufficiency and pulmonary embolism during the post partum period.

A 33 year old pregnant lady presented at 29 weeks of gestation with worsening headache. The MRI brain revealed an enlarged pituitary 14x11x9mm in size. There was no visual field defect and the relevant pituitary hormones were unremarkable, as were serum electrolytes and osmolality. She was observed closely without intervention. A follow up MRI at 35/40 showed further enlargement of the pituitary gland. The pituitary hormone abnormality detected then was low TSH at 0.02mU/L. The visual perimetry test revealed an upper quadrantanopia in the temporal field of the left eye and she was commenced on Cabergoline 0.5mg 3 times/ week.

She underwent elective cesarean section at term without any complications. At 8th weeks post partum a repeat MRI showed significant reduction in the size of the pituitary gland. Her Cabergoline was therefore ceased.

At 9-10 weeks postpartum, developed palpitations and extreme heat intolerance. TFTs showed FT4 27.2pmol/L, FT3 18.1pmol/L, TSH <0.01/L. TSH receptor antibody was negative, thyroid uptake scan later revealed normal 1^{131} uptake with possible quisent Grave's disease. She also had a positive family history of Grave's. She was started on Carbimazole, which was ceased after 2 months as her TFT‘s normalized.

She continued to lose significant weight and appetite despite treatment. Her ACTH level then was10ng/L (10-50) with low cortisol of <10nmol/L and she was treated with hydrocortisone. After about 10 days, she again presented with pleuritic chest pain and the CTPA revealed a filling defect with an incidental prominent thymic tissue. She was commenced on warfarin. During further follow up she remained asymptomatic, visual symptoms improved. She was maintained on physiological dose of hydrocortisone.

The following key points will be addressed in discussion: differential diagnosis of pituitary enlargement in pregnancy, clinical features and management of Lymphocytic Hypophysitis, association of prominent thymic tissue with thyrotoxicosis/ Grave's and hormonal predisposition to pulmonary embolism.

(1) Z Karaca. Pregnancy and pituitary disorders. European Journal of endocrinology. 162 453-475
(2) Joan-Andres Rivera. Lymphocytic hypophysitis: disease spectrum and approach to diagnosis and therapy. Pituiatry (2006) 9:35-45
(3) Van Nieuwkoop C.A. mediastinal mass: Grave’s disease related thymic hyperplasia. European Journal of Internal medicine. 2005; 16(8): 606-7
(4) Death from Thyrotoxicosis. Lancet Volume 302, Issue 7834, Pages 894-895
(5) B Corenblum. Pituitary disease and pregnancy. emedicine endocrinology
(6) M J Kupersmith. Visual loss in pregnant women with pituitary adenomas. Ann Intern med (1994)121; 7 473-477
(7) Y Ozawa. Recovery from lymphocytic hypophysitis associated with painless thyroiditis: clinical implications of circulating antipituitary antibodies.Euro J Endocrinology vol 128, Issue 6 493-498
(8) G Gemzell. Outcome of pregnancy in women with pituitary adenoma. Fert Steri. 1979;31:363-72
(9) P Caturegli. Autoimmune hypophysitis.Endocrine reviews. 26(5)599-614
(10) N G Dan.Pituitary apoplexy in association with lymphocytic hypophysitis.J Clin neurosc.2002;9(5)577-80

426

AN ATYPICAL CASE OF SITAGLIPTIN (JANUVIA) AND ATORVASTATIN RELATED PROXIMAL MYOPATHY

J. Tu[1], T. Berhane[1], R. Manasseh[2]

[1]Department of Rheumatology, Sir Charles Gairdner Hospital, Perth, WA, Australia
[2]Rheumatology, Suite 40-42, St John of God Hospital Murdoch, Perth, WA, Australia

Drug induced inflammatory myopathies are associated with significant morbidity and mortality. We report an atypical case of Sitagliptin (Januvia) and Atorvastatin related proximal myopathy. A 75 year old female presented with three months of progressive proximal muscle weakness and bilateral hip pain, coinciding with introduction of Sitagliptin therapy. Clincial features were suggestive of a drug induced myopathy, with normal inflammatory markers. Further autoimmune screening was negative and electromyography testing demonstrated changes consistent with a non-necrotizing myopathic process. Sitagliptin was stopped and there was a dramatic improvement in her symptoms. Using the Naranjo scale to calculate the likelihood that this adverse drug reaction was due to Sitagliptin, results suggested a possible relationship between the two variables. This case highlights that Sitagliptin is associated with an increased risk of developing proximal myopathy, especially in combination with statin therapy. In patients prescribed therapy wtih such newer diabetic agents, vigilance for complications including myalgia and rhabdomyolysis must be maintained.

427

HIGH GRADE IGF-II PRODUCING SARCOMA CAUSING RECURRENT HYPOGLYCEMIA IN A RECENTLY GRAVID 29 YEAR OLD WOMAN

T. Wong, C. White, K. Chen

Endocrinology, Prince of Wales Hospital, Randwick, NSW, Australia

A recently gravid, 29 year old woman without history of diabetes mellitus was referred to our inpatient endocrinology service at 12 weeks post-partum for recurrent symptomatic episodes of hypoglycaemia, in the context of a recently diagnosed undifferentiated spindle cell sarcoma without evidence of metastatic disease.

Following an overnight fast, serum glucose level fell to 3.1 mmol/L; C-peptide, insulin and IGF-1 levels were undetectable. IGF-II level was increased at 123nmol/L (NR 47-94), consistent with an IGF-II mediated non-islet cell tumour hypoglycaemia (NICTH). Subsequent immunohistochemical staining on the biopsy specimen was strongly positive for IGF-II. Although the mass could not be resected, there were no further episodes of hypoglycaemia after the first cycle of chemotherapy. She was prescribed prednisone at a daily dose 20mg to be commenced in the event of recurrence.

NICTH is a clinical syndrome of recurrent symptomatic hypoglycaemia classically occurring in large tumours of mesenchymal origin, most commonly due to increased tumour production of a larger precursor of IGF-II, termed "big-IGF-II". "Big-IGF-II" has enhanced insulin-like metabolic activity due to reduced affinity for binding proteins and this may also impair counter-regulatory responses to hypoglycaemia. An IGF2:IGF1 ratio of greater than 10, in the context of low glucose, insulin and c-peptide levels is diagnostic of NICTH.

Surgical resection of the tumour, leads to resolution of hypoglycaemic episodes and a decrease in circulating big-IGF-II levels. Palliative approaches include regular oral or enteral feeding and pharmacotherapy. Glucocorticoid therapy is the most effective pharmacological modality of treatment and decreases big-IGF-levels. Growth hormone therapy is also able to achieve normoglycaemia, however its predominant mechanism of action is counter-regulatory.

1. de Groot J, Rikhof B, van Doorn J, Bilo H, Alleman M, Honkoop A1, van der Graaf W. Non-islet cell tumour-induced hypoglycaemia: a review of the literature including two new cases. Endocrine-Related Cancer. 2007. 14: 979–993

428

GENDER DIFFERENCES IN HORMONAL OUTCOME FOLLOWING SURGERY FOR NON-FUNCTIONING PITUITARY ADENOMAS

C. Caputo, T. Sutherland, P. McNeill, K. NG, W. Inder

Endocrinology, Radiology and Neurosurgery, St Vincent's Hospital, Fitzroy, VIC, Australia

Non-functioning pituitary adenomas (NFPA) generally present with features of mass effect and/or hypopituitarism. Primary therapy is surgical, however little is known regarding factors which determine hormonal status in these patients. A retrospective analysis of the hormonal presentation and outcome in 125 NFPA cases was undertaken, to determine if gender differences exist in the prevalence of hormone deficiencies. Other factors examined included mode of presentation and tumour characteristics.

At presentation, 55% (69) were males and 45% (56) were females (19 pre-menopausal age). Hypopituitarism (defined as ≥2 hormone deficiencies) at diagnosis was more prevalent in males (48%) compared with females (28%) (RR 1.46, CI 1.03- 2.09, p=0.037). Cases presenting incidentally (30%, n=37) occurred predominantly in post-menopausal women (PostMPF, 49%) compared to males (23%) and premenopausal women (PreMPF, 16%), (p=0.008). Giant tumours (>3cm) and invasive tumours occurred more frequently in PostMPF (70%) and males (80%) compared to PreMPF (27%) (p=0.002) but the presence of hypopituitarism was not associated with tumour size (>3cm) or invasiveness.

Post operative hormonal data at 6 months was available for 83 cases (46 male, 27 PostMPF, 10 PreMPF). Overall, 35% of patients with pre-operative deficiencies had recovery of a pituitary axis (gonadal, thyroid and adrenal). Greatest recovery occurred in PreMPF, particularly of gonadotrophins (75% vs 28% for combined PostMPF/male, p=0.008). Most males and PostMPF who had hormone deficiencies pre operatively (83%) remained deficient. Males had a higher frequency of developing post-operative hormone deficiency compared to females and had significantly higher likelihood of hypopituitarism (50% vs 17%, p=0.002). Diabetes insipidus developed in 10%, with no gender difference.

In conclusion, there appear to be significant gender differences in hormonal function and outcome in patients presenting with NFPA. Women have a better hormonal outcome than men with lower rates of post-operative hypopituitarism, and in PreMPF, a higher rate of hormonal recovery.

429

EVALUATION OF 25 (OH) VITAMIN D STATUS IN CHRONIC KIDNEY DISEASE PATIENTS OF NORTH QUEENSLAND

S. Chaubey, V. Manickam, G. Kan, L. Roberts, K. S. Sangla

The Townsville Hospital, Townsville, Douglas, QLD, Australia

Background: There has been increase in chronic diseases with increasing longevity and modern lifestyle. Vitamin D insufficiency and deficiency is related to various clinical conditions and there has been new interest in the role of 25 hydorxy vitamin D { 25 (OH) D } which may be separate from 1,25 $(OH)_2$ vitamin D .There are no studies to assess vitamin D status in chronic kidney disease (CKD) patients of north Queensland.

Objective: To assess 25 (OH) D status in patients with chronic kidney disease availing outpatient and in patient services of a tertiary level regional hospital in the north Queensland .

Method: Data was collected from 131 CKD patients aged 22 years to 90 years. It had 87 patients from Caucasian, 40 patients from Aboriginal and Torres State Islander (ATSI) and 4 patients from Asian background. It included patients from all the stages of CKD. Among them a significant proportion of patients (67/131) were in stage 5 CKD while 30 /131 were in stage 4 CKD and 28/131 in stage 3 CKD. Only 6 patients were in stage 1 or 2 CKD. According to the Kidney Disease Outcomes and Quality Initiative guidelines, patients were assigned to the following 3 groups: group 1, with a sufficient25 (OH) D serum level (>75 nmol/L); group 2, with an insufficient level (25 to 75 nmol/L); and group 3, with severe deficiency (<25 nmol/L).

Results: 59 / 132 patients (44.7%) had abnormally low 25 (OH) D status. It included 31/ 41(75.6%) patients from ATSI, 27/ 87 (31.0%) patients from Caucasian and 1/4 (25.0%) patient from Asian background. In stage 5 CKD 25/31 (80.6%) patients from ATSI background and 13/32 (40.6%) patients from Caucasian background had abnormally low25 (OH) D values. Furthermore 5/42 (11.9%) patients from ATSI background had severe deficiency (< 25 nmol/l) in contrast to only 2/ 87 (2.3%) subjects from Caucasian background. It is notable

that significantly larger proportion of ATSI patients 31/41 (76%) and all of the patients of Asian origin were on renal replacement therapy (RRT) in comparison to Caucasian 26 /87 (30%) in our study and

Conclusions: Our study revealed that 25 (OH) D insufficiency/deficiency was widely prevalent among all stages of CKD but it was more common in stage 5 CKD and subjects with ATSI background. Further studies are needed to find out implications of vitamin D deficiency in this setting .

430

EFFICACY OF GLARGINE INSULIN IN TYPE 2 DIABETES - AN AUDIT OF CLINICAL PRACTICE USING AUDIT4

D. M. Hoffman

Software 4 Specialists, Kensington, NSW, Australia

Background: The availability of an increasing number of new compounds and classes of diabetic medication has led to an exponential increase in therapeutic pathways such that it is very difficult to know which patterns of treatment are the most effective. A rapid, efficient auditing system is helpful in objectively comparing therapeutic options. Aim: The present study evaluated the outcome of commencing glargine insulin both as initial therapy and as a substitute for older regimens, most commonly in place of bd mixed insulins. Data capture and analysis was performed using an electronic point of care clinical management program, Audit4 (Software 4 Specialists, Australia). Design: Data was extracted from all patients seen in the last 2 years in a single practitioner private endocrine practice - extraction time 23 seconds. In that period, 1452 patients were seen with Type 2 diabetes: 370 patients were initiated on glargine, 92 patients (mean age 57.0 y, 61 % male) were insulin naïve (IN) and the remaining 278 patients (mean age 61.0 y, 53 % male)were substituted glargine in place of other insulin regimens (SI) - most commonly replacing twice daily mixed insulin with single daily glargine.

	HbA1c % (SD) (IN)	Weight (SD) kg (IN)	HbA1c % (SD)(SI)	Weight kg (SD)(SI)
T=0	9.32 (1.26)	89.97 (21.58)	8.99 (1.40)	88.72 (17.68)
T=3 months	8.61 (1.29)	91.93 (22.16)	8.86 (1.38)	92.08 (20.31)
paired change	-0.60 (1.22)	1.68 (2.86)	-0.00 (1.08)	0.24 (2.25)

Findings: Three months after commencing glargine, there was a modest improvement in HbA1c and modest weight gain for insulin naïve patients; for those changed to Lantus from other regimens, there was no significant change in HbA1c or weight. Conclusion: 1. There was no objective benefit in terms of HbA1c or weight from changing patients from established insulin regimens to glargine regimens. Appropriate clinical audit software can be very effective in objectively assessing outcomes in every day clinical practice.

431

ASSESSMENT OF PRE- AND POST-FORTIFICATION IODINE STATUS IN THE WEST AUSTRALIAN POPULATION

A. E. Makepeace[1,2], J. Joseph[1], N. Hadlow[3], E. Rossi[1], E. Lim[1,2]

[1]Biochemistry, PathWest Laboratory Medicine, Nedlands, WA, Australia

[2]Endocrinology, Sir Charles Gairdner Hospital, Nedlands, WA, Australia

[3]Biochemistry, Western Diagnostic Pathology, Myaree, WA, Australia

Introduction: WHO recommended criteria for determining iodine status of a population are based on median urine iodine levels. Mandatory iodine fortification of bread commenced in Australia in October 2009 and we determined pre and post fortification urine iodine levels in three population groups.

Method: Analysis was performed by Inductively Coupled Plasma-Mass Spectrometry [ICP-MS] on a Varian 820MS with standardization by aqueous calibrators containing sodium thiosulphate to stabilize iodide. Ethics committee approval was obtained for testing random urine samples from first trimester pregnant women [327 pre- fortification], diabetics having spot urine albumin [132 pre- and 154 post-fortification] and subjects undergoing bone metabolism studies [105 pre- and 107 post-fortification]. WHO criteria for iodine replete status are a median urine iodine level >100 ug/L and less than 20% of the population <50 ug/L and for pregnant women a median >150 ug/L.

Results: Compared to the previous chemical method, urine iodine determination by ICP-MS is rapid, accurate and has reference method status. The Table shows pre and post fortification urine iodine levels according to WHO criteria.

	Pre-Fortification		Post-Fortification	
	Median (ug/L)	Deficient*	Median (ug/L)	Deficient*
Bone Studies	105	18%	126	8%
Diabetes	142	8%	167	5%
Pregnancy	181	11%	In progress	In progress

*: Percentage of the population <50 ug/L

Although all three groups met criteria for adequate iodine status, pre-fortification subjects undergoing bone studies were borderline deficient, with a median of 105 ug/L and 18% with levels <50 ug/L. Fortification had no effect on diabetic subjects where pre and post

fortification levels were essentially identical. Iodised salt used in fortification program may show losses of iodine due to high humidity or poor packaging.

Conclusions: Although all three groups were iodine replete, post-fortification iodine levels failed to show an expected large increase in the iodine status of the bone study and diabetes groups.

432

LOCALISATION, TRANSIENCY AND INDEPENDENCE OF HAIR CORTISOL RESPONSES TO A BRIEF PAIN STRESSOR

J. R. McFarlane, C. F. Sharpley, K. G. Kauter

Brain & Behaviour Research Group, Centre for Bioactive Discovery in Health and A, University of New England, Armidale, NSW, Australia

Steroid hormones including cortisol have been measured in hair from humans and animals in a number of studies spanning several decades. It has been assumed that the hormones are laid down within the hair as it grows and hence concentrations measured reflect an accumulation over time. Recent data reporting cortisol synthesis in hair follicles (Ito *et al.*, 2005) have shown the existence of a parallel "peripheral" HPA-axis. There is evidence from *in vitro* studies and single-observation comparisons between groups that cortisol from hair follicles reflects endocrine changes associated with stressor demands. Some studies have reported that cortisol concentration changes along the hair shaft (Kirschbaum et al 2009) while others have shown it does not (Davenport et al 2006), consequently further work is required to investigate the conflicting reports before hair can reliably be used as a non-invasive method of obtaining cortisol concentrations as a measure of stress. To help resolve this issue we performed a study to measure the cortisol response in hair before and after a transitory stressor. Eight males underwent the 1 min Cold Pressor Test on their preferred hand and had hair collected from the pain site and from the opposite leg, as well as salivary cortisol at regular intervals before and after the pain stressor. Cortisol in the hair from the arm tested was significantly higher than basal levels within minutes of the Cold Pressor Test and then rapidly declined to basal levels within a few minutes. Cortisol in the hair from the leg remained unaffected. Salivary cortisol rose to a peak 15-20 minutes after the test. The data indicate that cortisol response in hair to stressor demand appears to be (a) swift but transitory, (b) localized to the site of the demand and (c) independent of central HPA-axis activity.

(1) Davenport, M. D., et al (2006) Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. Gen. Comp. Endocrinol. 147, 255-261.

(2) Kirschbaum, C., et al (2009) Hair as a retrospective calendar of cortisol production—Increased cortisol incorporation into hair in the third trimester of pregnancy. Psychoneuroend. , 34, 32-37.

433

LOW TESTOSTERONE LEVELS IN TYPE 1 COMPARED TO TYPE 2 DIABETIC MEN

M. Ng Tang Fui[1], P. Nguyen[2], E. Giannatti[1,2], J. Zajac[1,2], M. Grossmann[1,2]

[1]Austin Health, Heidelberg, Australia

[2]University of Melbourne, Melbourne, VIC, Australia

Background: Population-based studies have consistently shown that 50% of ageing obese men with type 2 diabetes have low testosterone levels. The prevalence of low testosterone in men with type 1 diabetes is less clear.

Aim: To determine the prevalence of low testosterone levels in men with type 1 diabetes, compared to age-matched men with type 2 diabetes.

Design: Morning testosterone levels were measured on consecutive patients presenting to outpatients from January 2007-2010 as well as from age-matched patients with type 2 diabetes.

Results:

	T1DM	T2DM
Number	74	75
Age (y)	47 (18-88)	54 (30-86)
BMI (kg/m^2)	27 (19-39)	30 (22-59)
HbA1c (%)	7.5 (5.4-14.4)	7.3 (5.1-13.1)
Total Testosterone (nmol/l)	18.3	11.9
Calculated Free Testosterone (pmol/l)	290	241

Values given as median and range

The prevalence of low total and calculated free testosterone was 8% and 25% respectively in type 1 diabetic men, compared to 26% (total) and 41% (free) in men with type 2 diabetes. Total testosterone was inversely correlated with obesity (BMI) in both groups.

Conclusion: The lower prevalence of low testosterone levels in type 1 compared to type 2 diabetic men suggests that adiposity may be a major driver of the low testosterone-diabetes association.

The response to borderline-low testosterone levels in diabetic men with unequivocal symptoms and signs of hypogonadism should include lifestyle measures with emphasis on weight loss.

434

HYPERTHYROXINEMIA WITH NON SUPPRESSED TSH –A DIAGNOSTIC DILEMMA

P. Nigam, K. S. Sangla, Y. M. Tan

Dept of Endocrinology & Diabetes, The Townsville Hospital, Douglas, QLD, Australia

A 26 year old female was referred by her General practitioner at age 15 with abnormal thyroid function tests (TFT).

FT4 was 27.1pmol/L (11.6-23.2), FT3 - 8.3pmol/L (3.5-6.5) with inappropriately normal/non suppressed TSH of 2.63pmol/L (0.35-5.50). Apart from occasional palpitations, she had no other symptoms/signs. Thyroid antibodies and TSH receptor antibodies were normal. USS thyroid showed a homogenous echotexture with the right lobe measuring 5 x 2 x 1.6 cm and the left at 5 x 2.2 x2 cm . There was a 1.5 cm mixed echogenic nodule in the isthmus. Fine needle aspirate was inconclusive.Tc ^{99} pertechnetate scan indicated a photopenic isthmus nodule. The overall uptake was homogenous at 4.4% (NR 1.1 -4.0%).

She has a strong family history of similar abnormal TFT 's in multiple family members in at least three generations. Her problem was compounded by the finding of a 4.6 x 4.7 mm micro adenoma on the left half of the pituitary on MRI scan. Her sex hormone binding globulin (SHBG) was 35 nmol/L (40-90) and TRH stimulation test showed more than 3-fold rise in TSH . Free alpha glycoprotein subunit of TSH (α GSU) was not elevated making the possibility of TSH secreting pituitary adenoma less likely.

Familial thyroid hormone resistance was diagnosed as a cause of her euthyroid hyperthyroxinemia based on strong family history, low SHBG and normal α GSU. Further investigations including gene mapping for Thyroid hormone receptor beta mutation (3) are being considered. The value/relevance of gene mapping is debatable.

Three years ago, she underwent partial thyroidectomy for the cold nodule which turned out to be benign. She remains euthyroid and is planning a pregnancy.

Thyroid hormone resistance is described as an autosomal dominant disorder.(1, 2, 3) Our patient's family tree revealed autosomal dominant inheritance with variable penetrance, which is rare (3).

Discussion points:

1.Diagnostic considerations and the protocol to study this rare familial pathology

2.Is genetic screening really indicated? Especially in context of pregnancy?

3.Should these patients be monitored for metabolic consequences like cardiomyopathy and osteoporosis?

Key references:

1. Refetoff S,Weiss RE, Usala SJ. 1993 Endo Rev. 14:348-399

2. Weiss RE, Hayashi Y, Nagaya T, Petty KJ, Murata Y, Tunka H, Seo H, Refetoff S. 1996 . J Clin Endocrinol Metab. 81:4196-4203

3. Takashi Nagaya, Norman L. Eberhardt, JLarry Jameson. 1993Journal of Clinical Endocrinology and Metabolism

435

VON HIPPEL-LINDAU DISEASE PRESENTING AS RECURRENT AND MULTIPLE PHAEOCHROMOCYTOMA

P. Nigam, U. Malabu, K. S. Sangla, Y. M. Tan

Endocrinology & diabetes, The Townsville Hospital, douglas, QLD, Australia

KF is a 25 year-old Caucasian female, diagnosed at the age of 8, with bilateral adrenal phaeochromocytoma when she presented with non-surgical intestinal obstruction. She had left total and right partial adrenalectomies. Gene analysis at the age of 18 confirmed von-Hippel-Lindau (VHL) disease (1) showing small V84L (c.250G>C) mutation with no family history of the disease (2, 3).

Between the age of 10 and 20, she underwent 8 more surgeries (including complete right adrenalectomy) for recurrence of right adrenal and extra adrenal phaeochromocytoma in the abdomen. This was detected by her persistently elevated urinary noradrenaline and imaging, including CT and MRI scans, although MIBG scan was negative. She was also commenced on replacement cortisone acetate and fludrocortisone post surgery. In all, 17 tumours were excised.

Despite multiple excisions of recurrent phaeochromocytoma, her 24hr urinary noradrenaline and serum chromogranin A remained elevated. At age 25, one suspicious mediastinal and two right supraclavicular lesions were confirmed with PET/CT scan. Following surgical excision, her urinary noradrenaline and serum chromogranin A normalised (see table)

Biochemistry	Urinary noradrenaline (Normal50-600 nmol/d)	Urinary adrenaline (Normal <120nmol/d)	Urinary Normetadrenaline/creatinine ratio(Normal <0.25 mmol/mol)	Serum Chromogranin0 A (Normal<17.2U/L)
Pre-operative 2009	1300	<25	0.56	23
Post-operative 2010	500	74	0.15	12

She remains asymptomatic and normotensive. Ongoing surveillance for the components of the syndrome has so far been negative, with no evidence of cerebellar (4) or retinal hemangioblastoma or renal cyst/carcinoma.

Discussion points:

1. Use of biochemical markers and imaging (including the role of PET/CT) for ongoing surveillance

2. Management of her tumour recurrence both medically and surgically, including the role of tyrosine kinase inhibitors

References:

1. Maher E, Kaelin WG. 1997 von Hippel-Lindau disease. Medicine 76:381-391

2. Walther M.M., R. Reiter, H.R. Keiser et al. 1999. Clinical and genetic characterization of pheochromocytoma in von Hippel-Lindau families: J Urol 162: 659-664

3. Latif F, Tory K, Gnarra J, et al. 1993 Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 260:1317-1320

4.Wanebo JE, Lonser RR, Glenn GM, Oldfield EH. 2003 The natural history of hemangioblastomas of the central nervous system in patients with von Hippel-Lindau disease. J Neurosurg 98:82-94

436

DIETARY SODIUM INCREASES THE SEVERITY OF OBSTRUCTIVE SLEEP APNEA IN PATIENTS WITH ALDOSTERONE EXCESS AND RESISTANT HYPERTENSION

E. Pimenta[1], K. K. Gaddam[2], R. D. Gordon[1], S. Oparil[2], M. Stowasser[1], D. A. Calhoun[2]

[1]Endocrine Hypertension Research Centre, University of Queensland Medical School, Woolloongabba, Australia
[2]Vascular Biology and Hypertension Program, University of Alabama at Birmingham, Birmingham, AL, United States

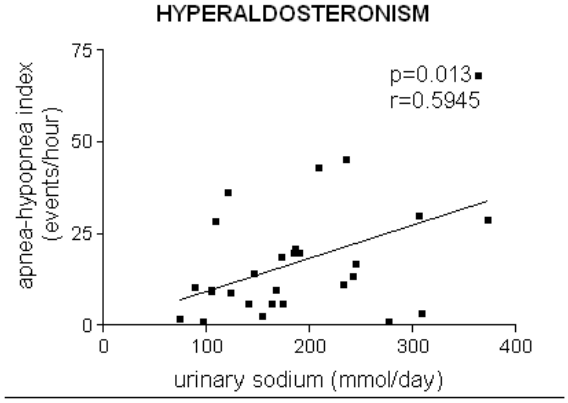
Background: Aldosterone excess promotes target organ deterioration and, in patients with resistant hypertension, is associated with severity of obstructive sleep apnea (OSA). Experimental and human data suggest that adverse cardiovascular and renal effects of aldosterone excess are dependent upon concomitant dietary salt intake.

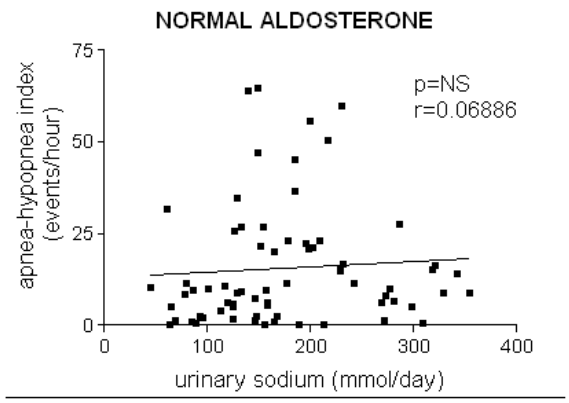
Objectives: Determine if dietary salt is related to severity of obstructive sleep apnea in patients with resistant hypertension and aldosterone excess.

Methods: Ninety-eight patients with resistant hypertension were prospectively evaluated with overnight polysomnography, plasma renin activity (PRA), 24-hour urinary excretion of aldosterone (UAldo) and sodium (UNa) while taking usual diet. Hyperaldosteronism was defined as PRA < 1 ng/mL/h and UAldo ≥ 12 µg/24-h .

Results The mean clinic blood pressure (BP) for all subjects was 156±22/89±13 mm Hg on an average 4.3±1.0 antihypertensive medications. There were no differences in systolic/diastolic BP, number of antihypertensive medications, age, duration of hypertension, body mass index and UNa between patients with hyperaldosteronism (n=29) and patients with normal aldosterone (n=69). A pnea-hypopnea index (AHI) strongly positively correlated with both UNa (r=0.5945, p=0.0007) and urinary aldo (r=0.4166, p=0.0246) in patients with hyperaldosteronism but not in patients with normal aldosterone (UNa, r=0.06886, p=NS; Ualdo , r=0.1001, p=NS).

Conclusions: These findings suggest that both aldosterone levels and dietary salt contribute to the severity of OSA in patients with resistant hypertension and hyperaldosteronism, but not in patients with normal aldosterone and similar BP levels. Our study supports treatment strategies based on reduction of aldosterone effects, by adrenalectomy or mineralocorticoid receptor blockade, in conjunction with low-salt diet in the treatment of OSA.





437

CARDIAC STRUCTURE IS LARGELY INFLUENCED BY DIETARY SALT IN PATIENTS WITH PRIMARY ALDOSTERONISM BUT NOT IN PATIENTS WITH NORMAL ALDOSTERONE

E. Pimenta, R. D. Gordon, A. H. Ahmed, D. Cowley, R. Leano, T. H. Marwick, M. Stowasser

Endocrine Hypertension Research Centre, University of Queensland Medical School, Woolloongabba, QLD, Australia

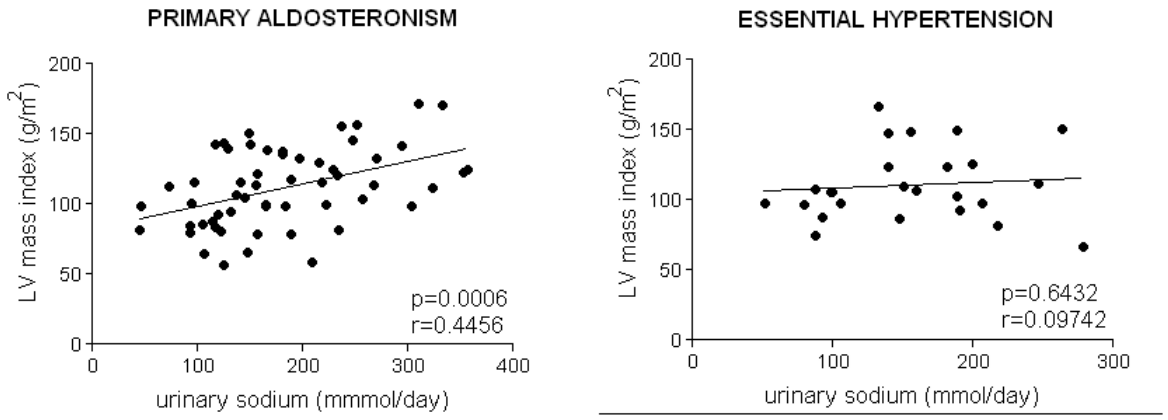
Background: Animal studies have demonstrated that dietary sodium intake plays a major role in the left ventricular (LV) hypertrophy and fibrosis induced by aldosterone excess.

Objectives: We sought relationships of aldosterone and dietary salt with LV structure and function in patients with primary aldosteronism (PAL) and essential hypertension (EHTN).

Methods: Subjects with confirmed PAL (n=60) and EHTN (n=25) were prospectively evaluated by echocardiography and 24h urinary sodium (UNa) excretion.

Results: In spite of similar 24h ambulatory blood pressure, PAL patients had significantly greater interventricular septum (IVS), posterior wall (PW), and LV end systolic and diastolic volumes than EHTN. There were no differences in LV mass (LVM), LVM index (LVMI) and ejection fraction between EHTN and PAL. While there were no significant correlations between aldosterone levels and echocardiographic measurements, UNa was strongly positively correlated with LVM, LVMI, IVS and PW in the PAL but not the EHTN group.

Conclusions: These findings emphasize the importance of dietary sodium in determining the degree of cardiac damage in PAL, and suggest that aldosterone excess may play a more permissive role. Dietary salt restriction might help reduce cardiovascular risk in patients with PAL.



438

A STUDY OF OSTEOPOROSIS IN PATIENTS ADMITTED TO HOSPITAL WITH LOW TRAUMA FRACTURE

A. Daniel[1], P. Davoren[2]

[1]Endocrinology, Royal Brisbane Hospital, Herston, QLD, Australia
[2]Endocrinology, Gold Coast Hospital, Southport, QLD, Australia

Background: A history of a fragility (low-trauma) fracture is an important risk factor for subsequent fracture in men and women (1, 2). We prospectively assessed patients admitted at the Gold Coast (level 3) Hospital with a low trauma fracture for Osteoporotic fracture risk with the intention of treating any modifiable risk factors as well as offering standard medical therapy for osteoporosis.

Methods: Patients admitted with a low-trauma fracture under the orthopaedics service in our hospital were screened clinically for risk factors predisposing to osteoporosis and were investigated for medical conditions predisposing to osteoporosis.

Result: There were a total of 81 patients enrolled in the study with a mean age of 79 years (SD=9.96); 59/81 (72.84%) were females. Fracture neck of femur was the predominant fracture type 66/81(81.48%). A significant proportion 60/81(74.07%; 95% CI 62.94% to 82.89%) of these patients had previous falls and 11/81 (13.58%; 95% CI 7.30% to 23.42%) were smokers. The medical disorders contributing to osteoporosis in our cohort of patients included rheumatoid arthritis 6/81 (7.4%; 95% CI 3.05% to 16.01%); chronic obstructive pulmonary disease 5/81 (6.17%; 95% CI 2.29% to 14.45%); diabetes 14/81 (17.28%; 95% CI 10.10% to 27.64%) chronic kidney disease 10/81 (12.34%; 95% CI 6.40% to 21.98%); previous hyperthyroidism 2/81 (2.46%;CI 0.43% to 9.46%); previous hyperparathyroidism 1/81 (1.23%; 95% CI 0.06% to 7.64%). Also 37/81 (45.68%; 95% CI 34.70% to 57.08%) had overt Vitamin D deficiency (<50 nMol/L) with 18/37 (48.65%; 95% CI 32.24% to 65.33%) associated secondary Hyperparathyroidism; 23/81 (28.39%; 95% CI 19.20% to 39.67%) patients had borderline Vitamin D levels (50-75 nMol/L) with 10/23 (43.47%; 95% CI 23.88% to 65.13%) associated secondary hyperparathyroidism; 20/22 (90.91% CI 69.38% to 98.41%) males had a suppressed testosterone but only 1/22(4.55%; 95% CI 0.24% to 24.88%) had a raised Luteinizing Hormone. Remarkably 20/81 (24.69%; 95% CI 16.08% to 35.75%) were previously diagnosed to have osteoporosis and they had significantly higher steroid use (Odds Ratio=8.16).

Conclusion: Our study indicates a high prevalence of overt and borderline vitamin D deficiency and secondary hyperparathyroidism in our cohort of patients with low trauma (fragility) fractures. A high prevalence of previous osteoporosis and recurrent falls were major contributors towards fragility fractures. Chronic steroid use and renal disease as well decreased calcium intake were other risk factors whilst smoking and alcohol use were not major contributors towards osteoporosis. The role of androgen deficiency in male patients needs to be further clarified.

1. Reedman KB, Kaplan FS, Bilker WB, et al. Treatment of Osteoporosis: are physicians missing an opportunity? J Bone Joint Surg Am 2000; 82-A: 1063-1070.
2. Harrington JT; Broy SB; Derosa AM; Licata AA; Shewmon. DA Hip fracture patients are not treated for osteoporosis: a call to action. Arthritis Rheum 2002 Dec 15;47(6):651-4.

439

IS TOTAL CALCIUM ADEQUATE FOR THE DIAGNOSIS OF PTH-DEPENDENT HYPERCALCAEMIA?

J. Ng[1,2], N. Kent[2], B. Stuckey[1], A. Makepeace[1,2], J. Walsh[1], G. Ong[1], E. Lim[1,2]

[1]Endocrinology, Sir Charles Gairdner Hospital, Nedlands, WA, Australia

[2]Clinical biochemistry, PathWest Laboratory Medicine, Perth, WA, Australia

Abstract

Introduction: Hypercalcaemia is usually diagnosed by elevation of total calcium. However, ionised calcium is the biologically active form and the major determinant of calcium homeostasis. In this study we aimed to identify the prevalence of normal total calcium amongst patients with PTH- dependent ionised hypercalcaemia.

Methods: All cases of total and ionised hypercalcaemia were identified from the database of fasting calcium metabolic studies between December 30 2005 and January 1 2008 to the Queen Elizabeth II Medical Centre PathWest laboratory. These studies are a panel of fasting morning blood and urine tests which include plasma total and serum ionised calcium, intact parathyroid hormone (PTH, DPC Immulite 2000 method), phosphate, 25-hydroxvitamin D (25-OHD, Diasorin RIA), creatinine, urinary calcium and phosphate indices and bone turnover markers. In those with hypercalcaemia (ionised calcium >1.32 mmol/L and / or total calcium >2.55 mmol/L) we defined a non-suppressed PTH as >5 pmol/L (ie above the mid-point of the reference range) and an elevated PTH to be >9 pmol/L (reference range 0.9-9.0pmol/L). Vitamin D deficiency was defined as <50nmol/L. Studies in which data on ionised or total calcium were unavailable were excluded from the analysis. Serial studies performed on the same patient were not excluded.

Results: Of the 6982 studies available 67 were excluded due to missing data, leaving 6915 for analysis. There were 534 cases of ionised and / or total hypercalcaemia. Ionised hypercalcaemia (corrected to pH 7.40) was present in 493 studies (92%) and in 250 of these (51%) the total calcium (adjusted for albumin) was not elevated. The range of total calcium was 2.28-3.92 mmol/L in those with ionised hypercalcaemia. Of the 291 studies with elevated total calcium, only 41(14%) had a normal ionised calcium. Further analysis stratifying according to PTH and vitamin D level is in the table below.

	Ionised hypercalcaemia (>1.32 mmol/L) with normal total calcium	Ionised (>1.32mmol/L) and total (>2.55mmol/L) hypercalcaemia	Elevated total calcium (>2.55mmolL) with normal ionised calcium
n=534	243 (45.5%)	250 (46.8%)	41 (7.7%)
PTH>5 n=446	204 (45.7%)	221 (49.6%)	21 (4%)
PTH>9 n=341	136 (39.9%)	193 (56.6%)	12 (3.5%)
Vit D <50 n=153	56 (36.6%)	85 (55.6%)	12 (7.8%)

Conclusion: We have confirmed that measurement of total calcium alone is inadequate for the biochemical diagnosis of hypercalcaemia and may result in misdiagnosis in 40-50% of cases. Thus serum ionised calcium provides a more accurate reflection of calcium homeostasis in PTH-dependent hypercalcaemia.

440

PARATHYROID HORMONE EXCESS AND DEFICIENCY: IS PETER REALLY ROBBED OR PAUL PAID?

T. D.T. Vu[1,2], A. Ghasem-Zadeh[2], X. Wang[2], Q. Wang[2], R. Zebaze[2], E. Seeman[1,2]

[1]Endocrinology, Austin Health, Heidelberg West, VIC, Australia

[2]Medicine, University of Melbourne, Heidelberg, VIC, Australia

Parathyroid hormone (PTH) excess is believed to produce cortical thinning by endocortical resorption while being anabolic at trabecular sites. However, the proposed anabolic effect may be spurious as high intracortical remodelling adjacent to the marrow causes cavitation in the cortex (trabecularization) leaving cortical remnants of trabecular appearance which may be erroneously measured as trabeculae of growth plate origin. Hence, determination of the effects of endogenous PTH excess requires separation of cortical remnants from trabeculae of growth plate origin.

We studied 11 patients with hypoparathyroidism (HypoP, 60.4+13.3years); 12 patients with untreated primary hyperparathyroidism (HyperP, 60.2+12.5years), and 11 patients with treated HyperP (56.7+16.0years). Images were acquired using high-resolution peripheral computed tomography at the radius and tibia and analyzed using new software (Strax1.0) which separates compact cortex from cortical remnants and quantifies porosity in the residual compact appearing cortex and the porosity trabecularizing the inner cortex. We determined the ratio of the compact cortex to the compact and trabecularized cortical mass (percentage compact cortex) which reflects the extent of trabecularization.

At the tibia, patients with untreated HyperP had 20% thinner cortices than patients with HypoP (0.80 vs 1.05mm, p=0.007) and a lower percent of compact cortex (74.98 vs. 77.09%, p=0.08). Intracortical porosity was 21% higher (10.9 vs. 8.55%, NS). There was no difference in trabecular density between HyperP and HypoP (109.53 vs 110.07mgHA/cc). Treated vs. untreated HyperP had lower porosity in compact (2.90 vs. 7.89%, p=0.018) and trabecularized cortex (5.7 vs. 10.90%, p=0.02) but the thickness of the compact cortex was not increased (0.80 vs 0.82mm) and percent compact cortex was unchanged (74.98 vs 75.33%). Similar trends were observed at the distal radius.

PTH excess is associated with high intracortical remodelling and porosity with no detectable benefit on trabecular bone or cortical thickness. Surgical treatment partly reverses the deleterious effects excess PTH.

441

BONE MINERAL DENSITY AND BODY COMPOSITION IN WOMEN WITH TURNER SYNDROME

M. Yee[2], F. Milat[2,5], A. J. Vincent[1,2,3], B. J. Strauss[1,4]

[1]Clinical Nutrition and Metabolism Unit, Southern Health, Clayton, VIC, Australia
[2]Menopause Unit, Southern Health, Clayton, VIC, Australia
[3]Jean Hailes Research Unit, Monash Institue Medical Research, Clayton, VIC, Australia
[4]Depts. of Medicine and Nutrition & Dietetics, Monash University, Clayton, VIC, Australia
[5]Dept of Endocrinology, Prince Henry's Institute, Clayton, VIC, Australia

Introduction: Turner Syndrome (TS) is a common chromosomal condition in females, affecting 1/2000 live female births. Low bone mineral density (BMD), increased fracture risk and obesity are reported in these women. It is unknown whether this is due to the chromosomal abnormality or ovarian failure or both. Vitamin D deficiency is a risk factor for low BMD and is also associated with increased body mass index (BMI).

Method: Retrospective cross sectional audit of body composition (n = 28) and BMD as assessed (n = 40) by dual Xray absorptiometry (DXA) scan in TS women. TS women aged < 18 years, and with BMI < 18.5 kg/m2 were excluded from analysis. Levels of 25OH VitD were assessed within 6 months of the DXA scan and determined as deficient (VDD, < 75 umol/L, n = 10) or replete (VDR, >= 75 umol/L, n=5).

Results: Mean age was 32.5 years (range 18,59). Mean BMI was 26.8 (19.5,42.7). Mean percent body fat mass was 36.9% (20.1,52.0). Mean appendicular lean tissue mass index (kg/m2, ALTMI) was 6.48 (4.96,8.21). Mean total body T score was -0.50 (-2.6, +1.8). Mean lumbar spine T score -1.10 (-4.1,+1.9) and mean L femoral neck T score -0.73 (-2.8,1.5).

Mean vitD level was 67 umol/L (30,115).

VDD women, compared to VDR women were not different with respect to BMI, % total body fat, or ALTMI. VDD women compared to VDR women were not different with respect to t scores for total body, lumbar spine or femoral neck BMD.

Conclusion: TS women have BMI in the overweight range and increased % body fat. Unlike other reports in more general populations, in TS women, vitamin D status did not relate to body composition or bone density.

442

CORRECTION OF ALDOSTERONE EXCESS BY ADRENALECTOMY REDUCES SALT APPETITE IN PATIENTS WITH ALDOSTERONE-PRODUCING ADENOMA

E. Pimenta, R. D. Gordon, A. H. Ahmed, D. Cowley, D. Robson, C. Kogovsek, M. Stowasser

Endocrine Hypertension Research Centre, University of Queensland Medical School, Woolloongabba, QLD, Australia

Background: Salt appetite is a motivated behavioural state that drives animals to seek and ingest foods and fluids that contain sodium. Experimental studies have demonstrated that aldosterone stimulates salt ingestion and some brainstem neurones are specifically aldosterone-sensitive. However, the role of aldosterone in determining salt appetite in humans is unknown.

Objectives: To explore the role of aldosterone in contributing to salt appetite in humans by evaluating patients with aldosterone-producing adenoma (APA) before and after adrenalectomy (ADX).

Methods: Fludrocortisone suppression testing (FST), which has a very strict protocol, is performed in order to definitively confirm or exclude primary aldosteronism. During FST, patients are admitted to ingest a high-salt diet (including Slow Na 30mmol tds and free access to dietary salt) and fludrocortisone for 5 days. Patients (n=21) with APA were admitted for FST before and 7.7 ±5.5 months after ADX. Urinary sodium (UNa) and volume (Uvol) measured on the last day (day 4) of FST were compared before and after ADX.

Results: P lasma aldosterone, aldosterone/renin ratio and urinary aldosterone significantly decreased and renin increased after ADX as expected. UNa and Uvol significantly decreased from 292.0±72.9 mmol/day to 241.5±46.4 mmol/day (p=0.0063) and from 2949±980.9 ml/day to 2064±574.2 ml/day (p=0.0003), respectively, despite equal salt supplementation.

Conclusions: Aldosterone excess in humans may contribute to salt appetite and its correction by ADX seems to reduce salt intake.

443

GRAVES' DISEASE AND VITAMIN D DEFICIENCY IN VIETNAMESE LIVING IN MELBOURNE

K. D. Truong[1,2,3], M. C. Matheson[4], J. M. Wentworth[5], R. P. Anderson[5], P. R. Ebeling[1,2,3]

[1]Medicine, The University of Melbourne, Melbourne, VIC, Australia
[2]Endocrinology, Western Health General Hospital, Melbourne, VIC, Australia
[3]Medicine, The Royal Melbourne Hospital, Melbourne, VIC, Australia
[4]The School of Population Health, VIC, Australia
[5]Walter and Eliza Hall Institute of Medical Research, Melbourne, VIC, Australia

BACKGROUND: There has been an increasingly high attendance rate of Vietnamese Graves' disease (GD) patients at Western Hospital, and local clinics in Melbourne. This suggests that a genetic or environmental factor leads to an increased susceptibility to GD, which could be specific to Vietnamese; one of the possible environmental factors could be Vitamin D deficiency.

AIMS: Our research aims to determine the HLA haplotypes within a group of Vietnamese patients with GD and Vietnamese control participants; and to determine the lifestyle factors that lead to the development of Vitamin D deficiency in Vietnamese. METHODS: The research is divided into two components. Component 1 involves GD patients and control participants, who will undergo HLA genotype analysis for HLA-Bw46, HLA-DQB1*0502 and HLA-DRB1*1602 alleles, to determine GD genetic inheritance and predisposing background. Component 2 will determine a new prevalence of Vitamin D deficiency that will confirm the high prevalence of Vitamin D deficiency previously discovered. A total of 180 participants will be recruited, including 60 GD patients and 120 control participants.

RESULTS: Results of our earlier study in 2008 revealed the prevalence of Vitamin D deficiency and insufficiency to be as high as 88% in Vietnamese living in Melbourne. This is the highest prevalence of Vitamin D deficiency ever recorded for any sample population of this size. A total of 158 out of 180 Vietnamese had Vitamin D levels <75 nmol/L. Results for this study are currently under investigation. CONCLUSION: For the first time, HLA profile associated with GD in Vietnamese would be reported, which will add to the existing knowledge of HLA genetics in Asians, to provide a clue to the aetiology and pathogenesis of GD. This will allow better understanding of the genetic background for GD, which could potentially lead to improved diagnosis and treatment methods.

444

SERUM ACTIVIN A AS AN INDEPENDENT PREDICTOR OF TYPE 2 DIABETES MELLITUS

H. Wu[1], M. Wu[2], Y. Chen[1], D. J. Phillips[3], M. P. Hedger[1]

[1]Monash Institute of Medical Research, Monash University, Clayton, 3168, Australia
[2]Jean Hailes Clinical Research Unit, School of Public Health,, Monash University, Clayton, VIC, Australia
[3]La Trobe University, Bundoora, VIC, Australia

Activins A and B are immunoregulatory cytokines that are elevated in many inflammatory diseases, and their activity is blocked by a specific binding protein, follistatin. Inflammation is implicated as an important aetiological factor in type 2 diabetes mellitus (T2DM). This study examined the relationship between activin A and B, follistatin and T2DM. A total of 92 participants (34 males, 58 female) aged between 50 and 75 years were recruited and fasting blood samples were collected. An oral glucose tolerance test (OGTT) was performed on participants who had not been diagnosed with T2DM. Blood glucose, insulin, HbA1c, cholesterol, HDL, LDL, triglycerides (TG), C-reactive protein (CRP), creatinine clearance and glomerular filtration rate were measured. Activin A and B were measured by ELISA and follistatin by radioimmunoassay. Insulin resistance was calculated using the homeostasis model assessment of insulin resistance [HOMA-IR] index. Analysis was done by Pearson correlation, one-way ANOVA and multiple regressions. Thirty-nine subjects had a normal OGTT, 17 subjects had impaired glucose tolerance and/or impaired fasting glucose and 36 subjects were diabetic. There were significant positive correlations between activin A and fasting glucose, insulin, HbA1c and HOMA-IR. Activin B was positively correlated with glucose, insulin, HOMA-IR and HbA1c. Follistatin was positive correlated with HOMA-IR, CRP and TG. After adjusting for BMI, gender, age, HDL, cholesterol, blood pressure and creatinine clearance using multiple regression, activin A and B were found to be independent positive predictors of fasting glucose. After adjustment for BMI, cholesterol, gender and age, there was a positive relationship between activin A and insulin, but no significant correlation between activin B and insulin. These data indicate that, in the population studied, activin A is an independent risk indicator for T2DM, and further studies to investigate the role of activin A and its binding protein follistatin in this disease may be valuable.

445

SERUM TOTAL OSTEOCALCIN LEVEL PREDICTS MORTALITY IN OLDER MEN. THE HEALTH IN MEN STUDY

B. B. Yeap[1,2], S. A.P. Chubb[1,3], L. Flicker[1,4], K. A. McCaul[4], P. R. Ebeling[5], J. P. Beilby[3], P. E. Norman[6]

[1]School of Medicine and Pharmacology, University of Western Australia, Perth, WA, Australia
[2]Department of Endocrinology and Diabetes, Fremantle Hospital, Western Australia, Fremantle, WA, Australia
[3]PathWest Laboratory Medicine, Fremantle and Sir Charles Gairdner Hospitals, Western Australia, Perth, WA, Australia
[4]WA Centre for Health and Ageing, University of Western Australia, Perth, WA, Australia

[5]*Department of Medicine, University of Melbourne, Melbourne, VIC, Australia*
[6]*School of Surgery, University of Western Australia, Perth, WA, Australia*

Introduction and aims: Bone-derived undercarboxylated osteocalcin regulates insulin secretion and sensitivity in mice, and reduced serum total osteocalcin (TOC) is associated with diabetes in humans. Recently we demonstrated an association between reduced TOC and metabolic syndrome in older men (1). We sought to test the hypothesis that reduced serum TOC predicts mortality from cardiovascular disease (CVD) during male ageing.

Participants and methods: Early morning sera from 4,047 community-dwelling men aged ≥70 years were assayed for TOC. Occurrence and causes of death were recorded via the Western Australian Data Linkage System covering the period from blood sampling in 2001-4 to 31 Dec 2009 giving 5-8 years follow-up. Cox regression analysis was performed to evaluate associations between TOC level at baseline and time to death, excluding men reporting bone fractures, Paget's disease, or bisphosphonate, glucocorticoid or warfarin use.

Results: There were 3,505 men included in the analysis which adjusted for age, BMI, waist:hip ratio, smoking, dyslipidemia, hypertension and medical comorbidities. There was a U-shaped association between TOC level and hazard ratio (HR) for overall mortality. Compared with men in the reference quintile (Q2), men in the lowest quintile of TOC values (Q1) had HR 1.39 (95% CI 1.08-1.79, p=0.01). Men in the highest quintile of TOC (Q5) had HR 1.45 (95% CI 1.14-1.84, p=0.002) compared with Q2.

Conclusions: Both lower and higher serum TOC levels predict mortality in older men. As lower serum TOC levels have been associated with metabolic syndrome in older men (1), reduced serum TOC may represent a biomarker or a contributor to mortality from cardiovascular causes. Further investigation is needed to clarify the association of higher serum TOC with mortality and to determine the potential scope for interventions which modulate TOC levels to preserve health in men.

(1) Yeap BB, et al. Eur J Endocrinol: in press, accepted 25 May 2010.

446

DIVERGENT ASSOCIATIONS OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS 1 AND 3 WITH MORTALITY IN OLDER MEN. THE HEALTH IN MEN STUDY

B. B. Yeap[1,2], S. A.P. Chubb[1,3], K. A. McCaul[4], K. K.Y. Ho[5], P. E. Norman[6], L. Flicker[1,4]

[1]School of Medicine and Pharmacology, University of Western Australia, Fremantle, WA, Australia
[2]Department of Endocrinology and Diabetes, Fremantle Hospital, Fremantle, WA, Australia
[3]Biochemistry, Fremantle and Sir Charles Gairdner Hospitals, PathWest Laboratory Medicine, Perth, WA, Australia
[4]WA Centre for Health and Ageing, University of Western Australia, Perth, WA, Australia
[5]Pituitary Research Unit, Garvan Institute of Medical Research, Sydney, WA, Australia
[6]School of Surgery, University of Western Australia, Perth, WA, Australia

Introduction and aims: Circulating insulin-like growth factor-I (IGF-I) interacts with binding proteins including IGFBP1 and IGFBP3, and IGF-I levels decline with increasing age. Levels of IGF-I and IGFBP3 are correlated while IGFBP1 is inversely associated with insulin resistance. However, the roles of IGF-I, IGFBP1 and IGFBP3 as predictors of ill-health in ageing men remain unclear. We examined associations between IGF-I, IGFBP1 and IGFBP3 with mortality in older men.

Participants and methods: This was a longitudinal study of 3,980 community-dwelling men aged ≥70 years resident in Perth, Western Australia. Plasma aliquots collected at baseline (2001-4) were assayed for IGF-I, IGFBP1 and IGFBP3 as previously reported (1). Occurrence and causes of death up to 2009 were ascertained via the Western Australian Data Linkage System. Cox regression analyses were performed to analyse associations between IGF-I and BP levels with time to death, adjusting for age, BMI, waist:hip ratio, smoking, dyslipidemia, hypertension and medical comorbidities.

Results: There was no apparent association between quintiles of IGF-I and overall mortality. Hazard ratio (HR) for death was greatest in men with IGFBP1 levels in the highest quintile of values (reference Q1: Q5 HR 1.6, 95% CI 1.2-2.0, p=0.001). By contrast, men with IGFBP3 in the lowest quintile experienced increased mortality, with HR for death lower in quintiles 2-5 compared with Q1 (reference Q1: Q5 HR 0.65, 95% CI 0.51-0.83, p=0.001). Higher IGFBP1 and lower IGFBP3 levels remained associated with mortality after added adjustment for IGF-I levels.

Conclusions: In older men, higher IGFBP1 and lower IGFBP3 levels predicted increased mortality, while IGF-I was not associated. Higher IGFBP1 influences mortality despite its inverse association with insulin resistance. Further research is needed to clarify whether IGFBP3 protects older men from dying via IGF-I-independent mechanisms.

(1) Yeap BB, et al. Eur J Endocrinol 2010;162:249-57.

447

INVESTIGATING ANDROGEN RECEPTOR-MEDIATED ANDROGEN ACTION IN THE NEUROENDOCRINE REGULATION OF OVULATION

X. Cheng, M. Jimenez, J. Spaliviero, C.M. Allan, D. Handelsman, K. Walters

Andrology, ANZAC Research Institute/University of Sydney, Sydney, NSW, Australia

Although recently the androgen receptor (AR) has been proven to have a role in female reproduction, its precise role in female reproduction is still not clear. We generated homozygous androgen resistant $AR^{-/-}$ female mice using Cre/LoxP recombination for an in-frame excision of exon 3, encoding the second zinc finger essential for DNA-binding. $AR^{-/-}$ females are sub-fertile with significantly reduced ovulation rates

(1) which is overcome by gonadotrophin hyperstimulation suggesting a defect in extra-ovarian regulatory mechanisms. Ovary transplantation studies confirmed that the sub-fertility is due to both intra-ovarian defects and a disruption in extra-ovarian hypothalamic-pituitary regulatory mechanisms. (2) Preliminary findings indicate no difference in LH levels between $AR^{+/+}$ and $AR^{-/-}$ mice at diestrus ($AR^{-/-}$: 0.3ng/ml ± 0.1; $AR^{+/+}$: 0.3ng/ml ± 0.1). However, at proestrus, although not significantly different, serum LH levels in $AR^{-/-}$ mice were lower ($AR^{-/-}$: 2.0ng/ml ± 1.1; $AR^{+/+}$: 4.4ng/ml ± 4.0, $n \geq 6$). To investigate further the role for AR-mediated actions in the positive and negative feedback regulation of ovulation, we have determined the optimal time to collect blood samples for the natural ovulatory LH surge as well as to evaluate negative and positive feedback. Evaluation of serum samples from $AR^{+/+}$ mice at proestrus collected between 16:00 and 19:30 confirmed that the optimum time to assess the natural ovulatory LH surge is 18:30. In order to evaluate estradiol negative and positive feedback on serum LH, we confirmed that ovariectomy plus the use of implants filled with 2µg of oestradiol over a 6 day period are optimal. Histological examination of haematoxylin and eosin stained pituitary sections revealed no overt structural abnormalities between $AR^{-/-}$ and $AR^{+/+}$ mice. In conclusion, we hypothesize that AR-mediated actions play a role in regulating ovulation via hypothalamic-pituitary regulatory mechanisms. Our preliminary findings support this hypothesis and we will use our optimized techniques to definitively investigate the precise role of AR-mediated androgen actions in the neuroendocrine regulation of ovulation.

(1) K. A. Walters et al., Endocrinology 148, 3674 (2007).
(2) K. A. Walters et al., Endocrinology 150, 3274 (2009).

448

KISSPEPTIN CELLS IN THE OVINE ARCUTE NUCLEUS EXPRESS PROLACTIN RECEPTOR (LONG-FORM) BUT NOT MELATONIN RECEPTOR (MT1)

Q. Li, I. J. Clarke, J. T. Smith

Physiology, Monash University, Clayton, VIC, Australia

Many species including sheep exhibit seasonality in reproduction. Melatonin, secreted at night by the pineal gland, transforms photoperiod to a humoral signal that regulates brain systems controlling reproduction. How melatonin influences reproductive function is not known. The circannual rhythmicity of a number of factors including prolactin is also regulated by photoperiod through changes in melatonin profile. Plasma prolactin levels in sheep are higher in the non-breeding season. Kisspeptin is synthesised from neurons in the ovine arcuate nucleus (ARC) and preoptic area and is a key stimulator of the hypothalamic-pituitary-gonadal (HPG) axis. Kisspeptin cells also mediate sex steroid feedback control of gonadotropin releasing hormone (GnRH) secretion. The expression of kisspeptin in the ARC is reduced during the non-breeding season. We hypothesized that kisspeptin expression is directly, or indirectly, regulated by melatonin and/or prolactin. We first examined the expression of melatonin receptor (MT1) in kisspeptin neurons in the ARC of ovariectomised (OVX) sheep using double-label *in situ* hybridization (ISH). Kisspeptin neurons were identified by digoxigenin-labelled riboprobes and MT1 neurons by radioactive ^{35}S-labelled riboprobes. No MT1 receptor expression was found on kisspeptin neurons, while strong MT1 mRNA expression was detected in the pars tuberalis. We then examined the expression of the long-form prolactin receptor (prlR) in ARC kisspeptin neurons of OVX sheep during the breeding and non-breeding seasons using double-label ISH. From a total of 992 kisspeptin neurons examined in 3 OVX ewes during the breeding season, 61.9±8% co-expressed prlR mRNA. Similarly, in 1111 kisspeptin neurons examined in 4 OVX ewes during the non-breeding season, 62.0±5% co-expressed prlR mRNA. Thus, ARC kisspeptin cells do not express MT1, but do express prlR, which may allow direct response to seasonal changes in prolactin levels. Studies are in progress to determine whether prolactin treatment during the breeding season regulates kisspeptin expression in the ARC.

Supported by NHMRC project grant (606538) and ARC Future Fellowship.

449

EFFECT OF OREXIN ON LH AND FSH SECRETIONS IN PRE-AND POST-PUBERTAL RAMS FED RESTRICTED DIET

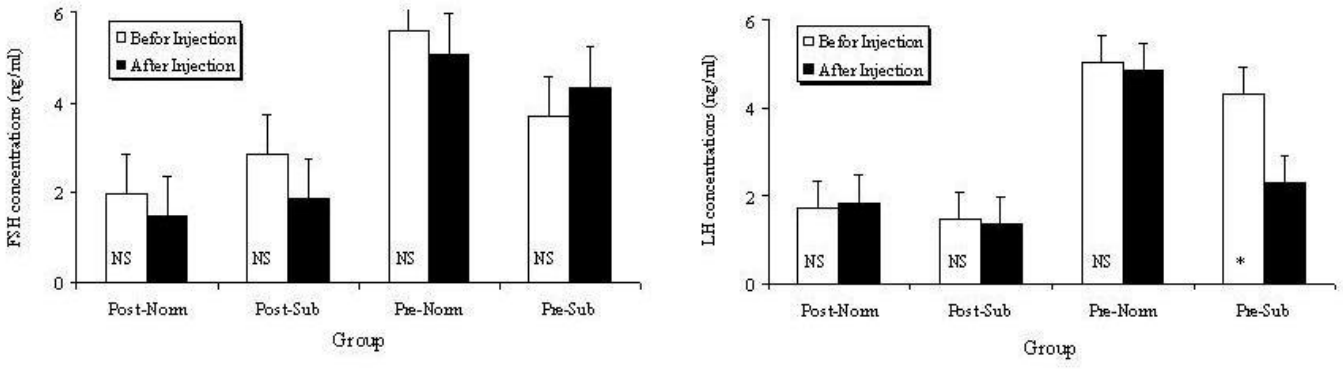
Z. Salarfar[2], H. Khazali[2], F. Moslemipur[1]

[1]Animal Sciences, Gonbad Higher Education Center, Gonbade Kavous, Iran
[2]Biological Sciences, Shahid Beheshti University, Tehran, Iran

The distribution of Orexin A neurons in the brain offers potential roles for orexin in the regulation of hypothalamo-pituitary-gonadal axis. The goal of the present study was to determine whether orexin affects Follicle-Stimulating Hormone (FSH) and Luterinizing Hormone (LH) secretions in pre- or pubertal rams under food restriction.

Twelve pubertal rams were catheterized and randomly divided into four groups; 10 pre-pubertal and 10 pubertal rams fed at either 100 or 50% of daily maintenance requirement for 5 consecutive days. Then, all animals were received 5 µg orexin A/kg BW via jugular vein. Blood samples were collected 4 hours before until 4 hours after the injection with 30min intervals and sera were assayed for LH and FSH concentrations via radioimmunoassay. Data were analyzed as a repeated measures design.

The injection of orexin A did not significantly affect FSH level in all groups. The injection of orexin A also had no significant effect on LH level, except in pre-pubertal ewes fed at sub-maintenance that a decrease in LH concentrations was observed.



Post: post-pubertal; Pre: pre-pubertal; Norm: normal feeding; Sub: sub-maintenance feeding

*NS: Not Significant; * p<0.05*

Results generally showed a decline in gonadotropins' levels that was reported in previous studies in rats (Pu *et al.* 1998; Barreiro *et al.* 2003). In general, our findings suggest that the effects of orexin on gonadotropins' secretions in rams can be potentially affected by pubertal and feeding conditions.

Keywords : Orexin A, FSH, LH, Puberty, Food Restriction

(1) Barreiro ML et al. 2003. Orexin 1 receptor messenger ribonucleic acid expression and stimulation of testosterone secretion by orexin A in rat testis. Endocrinology 145:2297-2306.

(2) Pu S et al. 1998. Orexin, a novel family of hypothalamic neuropeptides,modulate pituitary Lutrizing hormone secretion in an ovarian sterod-dependent manner. Regulatory Peptides 78:133-6.

450

INTRAVENOUS INJECTIONS OF NEUROPEPTIDE Y CAUSED HYPERTHYROIDISM IN MALE GOATS

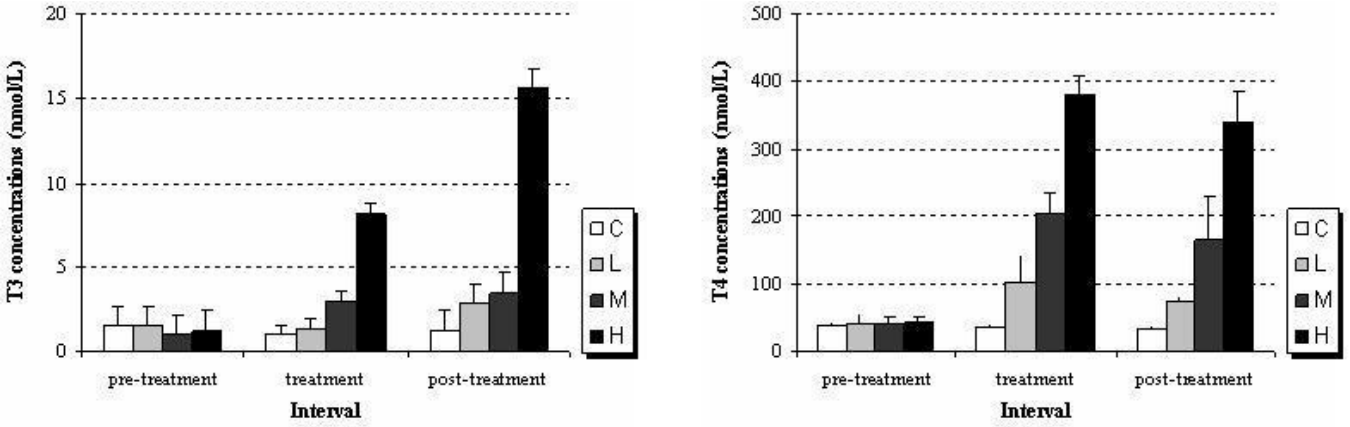
F. Moslemipur[1], S. Golzar Adabi[2]

[1]Animal Sciences, Gonbad Higher Education Center, Gonbade Kavous, Iran

[2]Animal Sciences, University of Ankara, Ankara, Turkey

Neuropeptide Y (NPY) is the most abundant peptide in mammals' brains affecting hypophysiotrapic factors secretion. Sixteen adult, male sannan goats (3 years old, weighing 45 ±3 kg) divided into four groups and then catheterized. Treatments were daily injections of 0 (control), 10, 20 and 40 µ g NPY/kg BW named C, L, M and H, respectively. The duration of experiment was consecutive 13 days; pre-treatment (days 1-3), treatment (days 4-10) and post-treatment (days 11-13) intervals. Injections performed in days of treatment interval. Blood samples were collected three times a day to determine triiodothyronine (T3) and thyroxine (T4) concentrations via radioimmunoassay.

Results showed that treatment H caused 11-fold increase in T_4 concentrations and 4-fold increase in T_3 concentrations vs. control ($p<0.001$). Treatment M increased both T4 and T3 concentrations while treatment L only increased T_4 concentrations ($p<0.01$). The stimulatory effect of NPY on thyroid hormones was transient; since they tended to decline in post-treatment interval but were yet significantly greater than control.



Previous studies in rodents demonstrated the inhibitory effect of NPY on thyroid hormones in human (Mihaly, et al. 2000) and rat (Wittmann, et al. 2002), but we found a marked stimulatory and dose-dependent effect of NPY on thyroid hormones in goat extended to post-treatmetn interval because of long thyroid hormones half-life. This discrepancy can be due to different methodology and differences between goat with rodents in neuroendocrine pathways controlling the activity of thyrotrop axis.

Key words: Neuropeptide Y - Thyroxine - Triiodothyronine – Goat

(1) Mihaly et al. 2000. Hypophysiotropic thyrotropin-releasing hormonesynthesizing neurons in the human hypothalamus ..., and á-melanocyte-stimulating hormone. J Clin Endocr Metab 85:2596-603.

(2) Wittmann et al. 2002. Adrenergic neurons contribute to neuropeptide Yergic innervation of hypophysiotrapic thyrotropin-releasing hormone synthesizing neurons in rats. Neurosci Lett 324:69-73.

451

ELEVATED LEPTIN LEVELS IN OBESITY INCREASE BLOOD PRESSURE BY SYMPATHO-EXCITATION

S. E. Simonds, R. D. Brown, P. J. Enriori, M. A. Cowley

Physiology, Monash University, Clayton, VIC, Australia

Obesity is associated with an increased risk of developing hypertension. Fat-derived leptin promotes weight loss by reducing appetite, and increasing energy expenditure through activation of the sympathetic nervous system (SNS). Although most obese humans and rodents have high plasma leptin levels, they are resistant to metabolic actions of leptin. This resistance appears to be selective to some regions of the hypothalamus(1,2). To test the hypothesis that stimulation of SNS by leptin causes an increase in blood pressure (BP) and heart rate (HR), mice fed a high fat diet (diet induced obese – DIO), regular chow (controls) and ob/ob (leptin deficient) were outfitted with radiotelemetry probes. Interscapular brown adipose tissue (iBAT) temperature was measured as a marker of sympathetic activity. iBAT was higher in DIO than controls and lower in ob/ob than controls ($p<0.001$). iBAT increased in DIO mice after peripheral or central leptin administration, despite no difference in caloric intake. DIO mice showed higher mean arterial pressure (MAP) (113.4 ± 1.5 vs 104.9 ± 1.6 mmHg, $p<0.001$), systolic BP (126.9±1.8 vs 118.8 ±1.8, $p<0.01$), diastolic BP (99.3±1.6 vs 91.2±2.8, $p<0.001$) and HR (579.8±7.28 vs 504.2±9.38, $p<0.001$) than lean controls. Leptin receptor antagonist (LRA) injection into the lateral ventricle of DIO mice (twice, daily) decreased MAP significantly after 7 days. Phosphorylation of STAT3, a marker of leptin action, was increased only in the dorso-medial hypothalamus (DMH) of DIO after leptin injection. This effect was prevented by previous treatment with LRA. A direct intra-DMH leptin receptor antagonist treatment also decreased MAP significantly after 7 days, suggesting the DMH is the region activated by leptin to stimulate the SNS. These results demonstrate that DIO mice are resistant to leptin's suppressive effect on appetite, but not on the sympathetic regulation of BP and HR. This strongly suggests the DMH mediates the sympathetic responses during hyperleptinemia.

(1) Enriori, P.J., et al. Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. Cell Metab 5, 181-194 (2007).

(2) Munzberg, H., Flier, J.S. & Bjorbaek, C. Region-specific leptin resistance within the hypothalamus of diet-induced obese mice. Endocrinology 145, 4880-4889 (2004).

452

SYSTEMS BIOLOGY APPROACH TO IDENTIFY NOVEL BIOMARKERS OF METABOLIC DISEASE IN OBESE CHILDREN UTILIZING NUCLEAR HORMONE RECEPTOR GENE EXPRESSION PROFILING.

S. Abd Rahman[1], N. Martel[1], N. Eriksson[1], R. Dahiya[2], M. Davis[3], G. E.O. Muscat[1], G. M. Leong[1,2]

[1]Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD, Australia

[2]Department Of Paediatric Endocrinology and Diabetes, Mater Children's Hospital, South Brisbane, QLD, Australia

[3]Queensland Facility for Advanced Bioinformatics, The University of Queensland, St Lucia, QLD, Australia

Introduction: Obesity is a global public health problem affecting up to 19% of children, increasing the risk of cardiovascular disease and Type 2 Diabetes Mellitus in later life. In the present study, we investigated the nuclear hormone receptor (NR) gene expression profiling of obese compared to non-obese pre-pubertal children (aged 6-12 years).

Methods: Peripheral blood samples were collected from 106 boys and girls. Analysis of NR mRNA gene expression from peripheral blood mononuclear cells (PBMCs) was performed in 51 age-matched boys (33 obese and 18 non-obese) and 55 age-matched girls (39 obese and 16 non-obese) using a NR Taqman low density array to assay the expression of the 48 Human NR genes. Statistical analysis was performed using the Statminer program.

Results: Thirty-two of the 48 NR genes were expressed in PBMCs of obese children. Six NR mRNAs (known to be involved in metabolism and two are orphan receptors) were differentially expressed in the obese versus non-obese children.

Conclusion: This study demonstrates that NR gene expression in PBMCs from obese children may provide novel insights into underlying mechanisms and risk for metabolic disease.

453

VALIDATION OF MULTI-FREQUENCY BIOELECTRICAL IMPEDANCE ANALYSIS (MFBIA) FOR MEASUREMENTS OF BODY COMPOSITION: A STUDY OF GROWTH HORMONE AND TESTOSTERONE ADMINISTRATION IN HEALTHY ADULTS

V. Birzniece, C. H. Khaw, U. J. Meinhardt, A. E. Nelson, K. K.Y. Ho

Pituitary Research Unit and Department of Endocrinology, Garvan Institute of Medical Research, St Vincent's Hospital, Darlinghurst, NSW, Australia

MFBIA is a convenient, non-invasive and inexpensive method for estimating body composition. It provides an indirect estimate based on the compartmentation of extracellular water (ECW) and intracellular water, from which fat free mass (FFM) and fat mass (FM) are derived. There is a paucity of data comparing MFBIA to established techniques. For the measurement of body composition, dual-energy X-ray absorptiometry (DXA) is a widely accepted method for estimating FM and FFM. FFM comprises body cell mass and ECW, which can be quantified by bromide dilution.

The aim was to compare estimates by MFBIA (Impedimed SFB7TM, Brisbane, Australia) of ECW, FM, and FFM against bromide dilution and DXA. Seventy-one healthy recreational athletes (43 men, 28 women; aged 18-40 years; BMI 24 ± 0.4 kg/m2) were studied in a double-

blinded, randomized, placebo-controlled design before and after treatment with growth hormone and testosterone (1). Data were analyzed using linear regression and the Bland-Altman method.

At baseline, there was a significant correlation between MFBIA and bromide dilution for ECW (r2 = 0.84, p < 0.001) and DXA for FM and FFM (r2 = 0.79 and 0.91, respectively; p < 0.001). ECW was 3.5 ± 1.1 % lower, FM was 22.4 ± 3.7 % lower and FFM was 13.7 ± 1.0 % higher with MFBIA compared to the established techniques (p<0.01). During the treatment, GH increased ECW and FFM and reduced FM; these changes correlated significantly between the methods (r2 for ECW, = 0.35; FM = 0.21; FFM = 0.61; p < 0.001). The change in ECW and FFM was not significantly different between the methods, however FM was 29.0 ± 8.8 % lower with MFBIA (p<0.01). In summary, good correlation was observed between MFBIA and the established techniques for measuring ECW, FM, and FFM. At baseline, MFBIA estimates of ECW were slightly and FM substantially lower, while FFM higher compared to bromide dilution and DXA. During the treatment, only FM estimated by MFBIA was lower compared to DXA. We conclude that MFBIA is an acceptable tool for measuring ECW, however it substantially under-estimates FM compared to DXA.

(1) Meinhardt et al., Ann Intern Med, 2010 May 4;152(9):568-77

454

IMPLICATION OF GENETIC VARIANTS NEAR *FTO, GNPDA2* AND *MC4R* WITH PERSISTENT CENTRAL OBESITY AND THE METABOLIC SYNDROME IN SOUTHERN CHINESE

C. Y.Y. Cheung[1], A. W.K. Tso[1], B. M.Y. Cheung[1], A. Xu[1], K. L. Ong[1], L. S.C. Law[1], N. M.S. Wat[1], E. D. Janus[3], P. C. Sham[2], K. S.L. Lam[1]

[1]Department of Medicine, The University of Hong Kong, Pokfulam,, China
[2]Department of Psychiatry, The University of Hong Kong, Pokfulam,, China
[3]Department of Medicine, University of Melbourne, Footscray, VIC, Australia

Objective: Central obesity plays an important role in the development of the metabolic syndrome (MetS) and is associated with increased risk of cardiovascular morbidity and mortality. Our group recently reported significant associations of three obesity-susceptibility single nucleotide polymorphisms (SNPs) identified from genome-wide association studies (1-2), including rs10938397 (GNPDA2), rs8050136 (FTO) and rs17782313 (MC4R), with general obesity and waist circumference in Southern Chinese (3). The major objective of this study was to further evaluate whether these SNPs could also predict the persistence of central obesity and MetS, in a 12-year longitudinal study.

Design and subjects: We conducted (i) a 12-year longitudinal study on persistent central obesity involving 354 subjects with persistent central obesity and 994 control subjects with persistent absence of central obesity, (ii) a MetS cross-sectional case-control study involving 816 cases and 1398 controls and (iii) a 12-year longitudinal study on persistent MetS involving 225 persistent MetS cases and 1221 controls with persistent absence of MetS, based on subjects from the population-based Hong Kong Cardiovascular Risk Factors Prevalence Study (CRISPS) cohort.

Results: Both FTO rs8050136 (one-tailed Page and sex-adjusted=9.5x10-3; OR(95%CI): [1.35(1.05,1.73)]) and GNPDA2 rs10938397 (one-tailed Page and sex-adjusted=1.5x10-3; OR(95%CI):[1.34(1.11,1.63)]) were significantly associated with persistent central obesity in the 12-year longitudinal study. In the MetS cross-sectional case-control study, the GNPDA2 rs10938397 was significantly associated with MetS (one-tailed Page and sex-adjusted=5.5x10-3, OR(95%CI):[1.20(1.04,1.38)]). However, none of these SNPs showed an individual association with persistent MetS. In the combined genetic risk analyses, each additional risk allele of the three SNPs were associated with 25% (95% CI:1.10,1.42; one-tailed Page and sex-adjusted=2.46x10-3) and 19% (95% CI:1.03,1.38; one-tailed Page and sex-adjusted=9.5x10-3) increased risk for persistent central obesity and persistent MetS, respectively.

Conclusion: This study suggests that the FTO and GNPDA2 variants may be useful for predicting persistent central obesity. Further functional studies of these genes are warranted.

(1) Thorleifsson G et al. Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. Nat Genet 2009; 41: 18-24.
(2) Willer CJ et al. Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. Nat Genet 2009; 41: 25-34.
(3) Cheung CY et al. Obesity susceptibility genetic variants identified from recent genome-wide association studies: implications in a chinese population. J Clin Endocrinol Metab 2010; 95: 1395-1403.

455

C-SKI TRANSGENIC MICE ARE PARTIALLY RESISTANT TO DIET-INDUCED OBESITY

M. Diaz[1,2], N. Martel[1,2], S. Myers[1], R. Fitzsimmons[1], M. Pearen[1], G. E.O. Muscat[1], G. M. Leong[1,2]

[1]Institute For Molecular Bioscience, University of Queensland, St Lucia, QLD, Australia
[2]Department of Paediatric Endocrinology and Diabetes, Mater Children's Hospital, South Brisbane, QLD, Australia

Transgenic mice over-expressing chicken Ski (c-Ski) develop marked muscle hypertrophy, and decrease in body fat. Previous studies in our laboratory suggest that the skeletal muscle expression of the master lipogenic regulator SREBP1c and the nuclear receptor, LXRα are suppressed in the Ski mice. Based on these and other findings, we hypothesized that Ski mice are resistant to diet-induced obesity. Wild type (WT) and Ski mice were challenged on a high fat (HF) diet for 12 weeks from 6 weeks old, weighed regularly, and glucose (GTT) and insulin tolerance tests (ITT) performed at 12-14 weeks old. At 18-20 weeks old body composition studies were undertaken and various metabolic parameters such as carbon dioxide production, oxygen consumption, food intake and activity investigated. The expression of metabolic and myogenic genes and those encoding nuclear hormone receptors (NRs), were assessed using custom designed ABI Taqman low density arrays (TLDA). Following the HF feeding regimen, both WT and Ski mice exhibited an increase in total body weight, however

within 2 weeks of HF feeding, WT mice gained almost 5% of their total body weight. In contrast, Ski mice gained just 0.8%. At 18 weeks, Ski mice displayed increased lean mass and 11% less body fat than WT mice, despite a lower metabolic rate, activity level and similar food intake to WT mice. Interestingly, Ski mice are mildly glucose intolerant on chow and HF diet. Ski mice are insulin sensitive on chow diet, yet both WT and Ski mice are insulin insensitive on HF diet. mRNA expression in Ski mice revealed changes in several key NR genes and gene pathways involved in glucose and lipid metabolism. These studies suggest that c-Ski transgenic mice are partially resistant to diet-induced obesity and that Ski targets several key NR and metabolic gene pathways to modulate body composition and metabolism.

456

A PARALLEL CIRCUIT IN GLUCOSE HOMEOSTASIS: A-MELANOCORTIN STIMULATING HORMONE FROM PITUITARY ENHANCES GLUCOSE UPTAKE BY SKELETAL MUSCLE

P. Enriori[1], A. Evans[2], S. Williams[1], B. Grayson[2], M. Garcia-Rudaz[2], C. Roth[4], T. Reinehr[4], U. Gebhardt[5], H. Müller[5], S. Simonds[1], M. J. Watt[1], B. A. Henry[1], R. D. Brown[1], I. J. Clarke[1], K. L. Grove[2], M. A. Cowley[1]

[1]Physiology, Monash University, Clayton, VIC, Australia
[2]Neuroscience, Oregon Health and Science University, Beaverton, Oregon, United States
[3]Endocrinology, Seattle Children's Hospital Research Institute, Seattle, WA, United States
[4]Paediatrics, Vestische Children Hospital Datteln, Witeen/Herdecke, Germany
[5]Paediatrics, Klinikum Oldenburg GmbH, Oldenburg, Germany

Central melanocortin pathways are well-established in regulation of energy balance and glucose homeostasis. However, scant data exists about the relevance of systemic melanocortin peptides. By studying healthy human subjects and patients who have hypopituitarism or after craniopharyngioma surgery, we demonstrated that α-MSH from the pituitary is the main contributor to circulating α-MSH in humans. Indeed, both patient groups had almost non-detectable α-MSH levels. We also found, by in vitro and in vivo experiments (measurement of α-MSH levels during a glucose tolerance test-GTT-) done in humans, monkeys and mice that α-MSH secretion is significantly increased by glucose and insulin. Using an established model of diet-induced entrainment of a thermogenic response we found that α-MSH infusion can produce a direct thermogenic response in sheep muscle. And we demonstrated that peripheral α-MSH infusion during GTT increases glucose disposal in lean but not in obese mice, and that effect was not prevented by previous icv treatment with AgRP, an MC4R antagonist, suggesting a direct effect on muscle. We then confirmed that effect was direct intramyocellular in origin because α-MSH was able to stimulate glucose uptake into isolated biopsies of soleus muscle from lean mice. Interesting, there is not any effect of α-MSH nor insulin in biopsies from obese mice. MC5R, but not the other subtypes were highly expressed in muscles and α-MSH treatment markedly increased cAMP levels and AMPK phosphorylation, suggesting that α-MSH actions are mediated by this signalling pathway. These results suggest that pituitary secretion of α-MSH after a meal may mediate post-prandial thermogenesis, and be part of a parallel system to supplement insulin mediated glucose uptake into skeletal muscle. The failure of α-MSH to act in muscles of obese insulin resistant mice suggest that failures of α-MSH mediated glucose uptake may contribute to diabetes in some model systems.

457

PLASMA LEVELS OF THE ATHEROGENIC MOLECULES, SOLUBLE CD40 LIGAND, P-SELECTIN AND VON-WILLEBRAND FACTOR IN SUBJECTS WITH IMPAIRED GLUCOSE TOLERANCE

M. Erbil

Clinical Biochemistry, Gulhane Military Medical Academy and School of Medicine, Ankara, Turkey

Introduction: Soluble CD40 ligand (sCD40L), soluble P-selectin (sP-selectin), and von Willebrand factor (vWF) are well known markers of platelet activation and endothelial function. In addition, they have important roles in the development and progression of atherosclerosis. Impaired Glucose Tolerance (IGT) confers an increased risk for type 2 diabetes mellitus and also cardiovascular disease. The aim of this study was to investigate circulating levels of sCD40L, sP-selectin and vWF in subjects with IGT who have no additional metabolic confounders such as hypertension, dyslipidemia and morbid obesity.

Methods: Plasma levels of sCD40L, sP-selectin and vWF were measured by ELISA method in 77 subjects with IGT and age, sex and body mass index matched 81 healthy controls with Normal Glucose Tolerance (NGT). High sensitive C reactive protein (hsCRP) level was determined in serum by turbidimetric fixed rate method by an automated analyzer. The degree of insulin resistance was determined by the homeostatic model assessment of IR.

Results: IGT and NGT groups were comparable for the demographic and anthropometric variables whereas, metabolic syndrome was more prevalent in the IGT group. No significant difference was observed between IGT and NGT subjects regarding the sCD40L, sP-selectin, vWF and hsCRP levels. However, IGT subjects with metabolic syndrome had significantly higher levels of sCD40L compared to both NGT subjects and IGT subjects without MS ($p<0.05$, for each) .

Conclusion: In the absence of other metabolic risk factors IGT per se is not associated with disturbance of platelet activation and also endothelial dysfunction.

458

CANDIDATE BASED EXPRESSION PROFILING OF A MUSCLE SPECIFIC NR4A3 TRANSGENIC MOUSE MODEL

N. Eriksson, M. Pearen, R. Fitzsimmons, G. E.O. Muscat

Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD, Australia

Nuclear receptors (NR) are hormone dependent transcription factors that translate physiological signals into gene regulation. Orphan NRs (including the NR4A subgroup) that lack identified ligands provide an opportunity for the discovery of unique signaling pathways. NRs control metabolism, and many of the 'orphans' are expressed in major mass tissues with burdensome energy loads, suggesting a role in energy homeostasis. Muscle accounts for ~40% of total energy expenditure and is the foremost site for fatty acid and glucose oxidation. NRs and skeletal muscle play a role in body weight, insulin sensitivity and the lipid profile.

Targeted silencing of Nr4a1/Nur77 and Nr4a3/Nor-1in skeletal muscle cells has been shown to modulate expression of genes that regulate fatty acid and glucose metabolism. Further studies concluded that Nor-1 is necessary for aerobic/oxidative metabolism. We are currently investigating the effects of muscle specific over-expression of Nor-1 in a transgenic mouse model. We have utilized quantitative real-time PCR to profile the expression of the entire NR superfamily, critical metabolic genes and several regulators of muscle growth, differentiation and mass. Nor-1 was found to be differentially expressed ~8 fold higher in the transgenic mice, with a concordant decrease in Nur77 expression. Interestingly, Nur77 null mice demonstrate a compensatory increase in Nor-1 expression, and on a high fat diet display weight gain and insulin resistance in skeletal muscle. Identification of novel Nor-1 targets that regulate metabolism in muscle may provide a platform for elucidating the phenotype of these mice.

459

SKELETAL MUSCLE 11BHSD1 ACTIVITY IS NOT INFLUENCED BY CENTRAL OBESITY OR INSULIN RESISTANCE IN NON-DIABETIC SUBJECTS.

W. Inder[1,2], V. Obeyesekere[1], F. Alford[1,2], C. Jang[1]

[1]Endocrinology and Diabetes, St Vincent's Hospital, Fitzroy, VIC, Australia

[2]Medicine, University of Melbourne, Melbourne, VIC, Australia

Local activation of glucocorticoids in insulin target tissues by the enzyme 11βhydroxysteroid dehydrogenase type 1 (11βHSD1) has been implicated in the aetiology of the metabolic syndrome. In obesity, 11βHSD1 is upregulated in adipose tissue, leading to the generation of higher tissue levels of cortisol which may increase insulin resistance. However, skeletal muscle (SkM) is the predominant site of insulin-mediated glucose disposal, which is known to be reduced in obesity. We aimed to determine if there is any relationship between SkM 11βHSD1 and markers of central adiposity and insulin resistance in non-diabetic subjects. Twenty non-diabetic volunteers (8 M and 12 F, mean age 55±13 years, BMI 21.5 to 47.6, mean 30.4±1.6 kg/m^2) underwent a single fasting blood sample followed by a muscle biopsy of vastus lateralis under local anaesthetic. Fasting glucose, insulin and adiponectin were measured in serum. SkM 11βHSD1 oxoreductase activity was determined by measuring the conversion of radiolabelled ^{3}H cortisone to cortisol by thin layer chromatography.

There was no correlation between BMI or waist circumference and 11βHSD1 activity or between HOMA and 11βHSD1 activity. When subjects were categorised according to abdominal obesity (waist circumference ≥ 102cm in men, ≥88cm in women), there was no difference between the groups in SkM 11βHSD1 activity.

SkM 11βHSD1 oxoreductase activity is not influenced by central obesity or insulin resistance in non-diabetic subjects. It is therefore unlikely that the insulin resistance observed in skeletal muscle of people with obesity is mediated by alterations in 11βHSD1.

460

STREPTOZOTOCIN-INDUCED HYPOINSULINEMIA CAUSES ENDOCRINE CHANGES IN MALE SHEEP

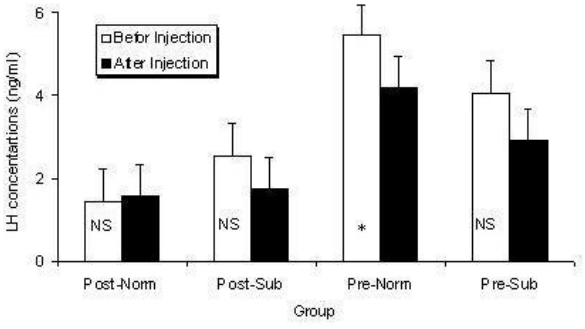
F. Moslemipur

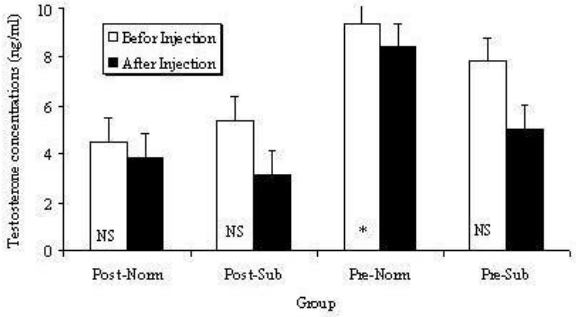
Animal Sciences, Gonbad Higher Education Center, Gonbade Kavous, Iran

Hypoinsulinemia is suitable condition to investigate insulin's endocrine roles. In this study, eighteen male Zel lambs (4 months of age, weighing 19.4±1.6 kg) divided into three groups, fed the same ration individually and catheterized for 8 weeks. Treatments were single intravenous injection of doses 0 (control), 25 and 50 mg/kg BW of streptozotocin named C, L and H, respectively. Fasted blood samples were collected twice weekly to assay insulin, leptin, growth hormone (GH) and IGF-1 concentrations via radioimmunoassay. Data were analyzed as repeated measures design.

Results showed that hypoinsulinemia occurred in group H with significant decline in insulin level in weeks 2, 4, 5 and 6 vs. control. Leptin concentrations in group H were significantly lower than control, except in week 5. In all weeks, GH concentrations in group H showed a marked decrease vs. control. Furthermore, IGF-1 concentrations in group H tended to decrease as compared to control, except in first and end weeks. Differences between group L and control were usually non-significant because of lake of hypoinsulinemia induction.

Hormone / Week		W1	W2	W3	W4	W5	W6	S.E.
Insulin (μIU/ml)	C	5.81^{b}	12.53^{a}	3.29	11.04^{a}	5.63^{a}	10.91^{a}	2.13
	L	5.90^{b}	11.50^{a}	3.46	10.10^{a}	5.47^{a}	11.50^{a}	
	H	6.83^{a}	6.60^{b}	4.05	7.16^{b}	4.41^{b}	7.84^{b}	
Leptin (ng/ml)	C	0.414^{b}	0.733^{a}	0.416^{b}	0.569^{a}	0.693^{a}	0.630^{a}	0.07
	L	0.469^{a}	0.635^{a}	0.632^{a}	0.533^{a}	0.395^{b}	0.516^{b}	
	H	0.365^{b}	0.409^{b}	0.602^{a}	0.394^{b}	0.300^{c}	0.406^{c}	
GH (ng/ml)	C	3.75^{a}	3.93^{b}	4.20^{a}	4.44^{a}	4.04^{a}	4.18^{a}	0.53
	L	3.63^{a}	4.86^{a}	4.88^{a}	4.58^{a}	4.11^{a}	4.56^{a}	
	H	2.50^{b}	3.03^{c}	2.82^{b}	2.36^{b}	2.71^{b}	2.91^{b}	
IGF-1 (ng/ml)	C	317.4^{b}	987.6^{a}	677.0^{a}	783.2^{a}	853.0^{a}	703.6	94.1
	L	349.8^{b}	928.2^{a}	625.4^{a}	837.8^{a}	601.6^{b}	660.4	
	H	397.0^{a}	535.6^{b}	449.6^{b}	478.8^{b}	451.0^{b}	677.0	





In each column, means with different superscript letters are statistically different ($p<0.05$)

Hypoleptinemia were shown in parallel with hypoinsulinemia in rat (Akirav *et al*. 2004), that has congruity with our finding. Conflicting effects were reported for insulin role in GH secretion (Ortiz-Caro *et al*. 1984; Masaoka *et al*. 2003), while we found they are positively correlated. Marked hypoleptinemia in this study may be a reason (Nagatani *et al*. 2000). Decrease in IGF-1 was natural phenomenon because of decreased GH level (Leon *et al*. 2004). In general, hypoinsulinemia can decrease leptin, GH and IGF-1 levels in sheep.

(1) Akirav et al. 2004. Partial leptin restoration increases hypothalamic-pituitary-adrenal activity while diminishing weight loss and hyperphagia in streptozotocin diabetic rats. Metabolism 53:1558-1564.

(2) Leon et al. 2004. Plasma concentrations of leptin, insulin-like growth factor-I, and insulin in relation to changes in body condition score in heifers. J. Anim. Sci. 2004. 82: 445-451.

(3) Masaoka et al. 2003. Enhanced plasma ghrelin levels in rats with streptozotocin-induced diabetes. FEBS Lett. 541:64-68.

(4) Nagatani et al. 2000. Leptin regulates pulsatile luteinizing hormone and growth hormone secretion in sheep. Endocrinology 141:3965-3975.

(5) Ortiz-Caro et al 1984. Diurnal variations of plasma growth hormone, thyrotropin, thyroxine, and triiodothyronine in streptozotocin-diabetic and food-restricted rats. Endocrinology 115:2227-2232.

461

EFFECTS OF HIGH FAT DIET STRESS ON VITAMIN D RECEPTOR DEFICIENT MICE

B. Ng[1], S. Lau[1], R. Stokes[1], R. Clifton-Bligh[2], J. E. Gunton[1]

[1]Garvan Institute, Sydney, NSW, Australia

[2]Kolling Institute, Sydney, NSW, Australia

Multiple studies implicate vitamin D signalling in glucose metabolism. Impaired insulin secretion and glucose tolerance have been reported in mice with mutant vitamin D receptor (VDRKO) (1). Worse glucose tolerance in the Demay VDRKO mouse-model with ablated VDR DNA-binding domain has not been demonstrated. We subjected these mice to beta-cell stress, in the form of high-fat feeding to determine if a beta-cell defect could be unmasked.

21 VDRKO, 19 heterozygous and 12 WT mice were fed "rescue" diet (2% calcium, 1.25% phosphorus, 20% lactose) from weaning. After base-line intraperitoneal glucose tolerance test (IPGTT) at 7 weeks, "rescue" high fat diet (HFD) (46.5% energy from fat) feeding was commenced. IPGTT was repeated after 4 and 9 weeks of high-fat feeding. Insulin tolerance testing and DEXA scanning was performed after 10 and 11 weeks of HFD, followed by sacrifice for organ weights and measurement of β-cell mass by histology.

Results: Female VDRKO exhibited the greatest deterioration in glucose tolerance (Table 1). The change in BGL was significantly higher in VDRKO versus heterozygote mice at the 0, 15, 30 and 60 minute time points, but there was no significant difference between VDRKO and WTs. Area under the curve analysis reveal worst insulin tolerance in VDRKO females (4310 vs 3300 for heterozygotes, 2616 for WT) and males (4834 vs 3455 for heterozygotes, 4164 for WT). DEXA revealed significantly lower bone mineral content in female (-0.0665±0.0217g/cm; p=0.007) and male (-0.061±0.029g/cm;p=0.051) VDRKO versus WT. Female VDRKO also had significantly less subcutaneous fat (-1.1±0.3%;p=0.005) and heavier livers (+0.9±0.3%;p=0.013) than WT. β-cell mass did not differ significantly.

Conclusions: VDR knockout has diverse effects on fat mass, insulin sensitivity and bone which could independently and differentially alter glucose tolerance, confounding the ability to demonstrate a specific beta-cell defect. A partial defect in vitamin-D signalling in the heterozygote state appears to protect from HFD-induced glucose intolerance.

Table 1: Average change in glucose tolerance from 7 to 16 weeks

Sex	Genotype	7-16week Average change in blood glucose (mmol/L) per IPGTT time point					
		0 min	15 min	30 min	60 min	90 min	120 min
Female	KO	+0.9±0.5	+5.3±1.9 *	+7.6±2.0 *	+6.3±1.6 *	+1.4±1.3	-0.6±0.9
	Het	-0.5±0.5	-2.6±2.1	-0.8±2.4	+1.2±1.7	-0.4±0.9	-0.9±0.8
	WT	-0.5±0.8	+1.8±2.3	+3.8±3.4	+6.5±3.5	+3.7±3.5	+2.9±3.1
Male	KO	+0.4±0.6	+1.2±2.0	+4.5±2.4	+3.9±2.1	+3.9±1.5 *	+1.8±1.3
	Het	-1.1±0.5	-1.8±2.4	-1.4±2.4	-0.3±2.5	-0.6±2.6	-1.5±2.5
	WT	+0.5±0.6	+2.0±1.2	+3.3±1.6	+2.9±2.8	+0.2±1.9	+0.8±1.9

*The mean difference between 7 week and 16 week GTT value is significant at the 0.05 level with paired t-test

(1) Zeitz U, Weber K, Soegiarto DW, Wolf E, Balling R, Erben RG: Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. The FASEB Journal 17:509-511, 2003

462

THE NUCLEAR HORMONE RECEPTOR, NOR-1 (NR4A3), A TARGET OF B-ADRENORECEPTOR SIGNALLING, CONTROLS INSULIN SENSITIVITY AND ENERGY HOMEOSTASIS IN SKELETAL MUSCLE

M. A. Pearen, R. L. Fitzsimmons, N. Eriksson, M. S.C. Wang, G. E.O. Muscat

Institute for Molecular Bioscience, University of Queensland, St Lucia, QLD, Australia

The NR4A subgroup of orphan nuclear receptors consists of three members in mammals (Nur77, Nurr1 and Nor-1). We have previously demonstrated that β-adrenoceptor agonists markedly induced the expression of the mRNAs encoding all three members of this subgroup in skeletal muscle (in vitro and in vivo) (Pearen MA et al 2006 Endocrinology 147, 5217-; Pearen MA et al 2008, Endocrinology 149, 2853-). Furthermore, we have previously knocked-down the expression of NR4A3/Nor-1 using siRNA in skeletal muscle cells (in vitro). This resulted in decreased palmitate oxidation and increased lactate accumulation consistent with a shift to anaerobic metabolism. Candidate-based mRNA expression profiling indicated that attenuation of Nor-1 altered the expression of genes that activate fatty acid oxidation and aerobic utilisation of pyruvate (Pearen MA et al 2008, Endocrinology 149, 2853-). To investigate the in vivo functional role of Nor-1 in skeletal muscle, we produced a transgenic mouse line that has over-expressed activated Nor-1 in skeletal muscle [by using the human skeletal alpha actin (HSA) promoter]. Analysis of this mouse model suggests that over-expression of Nor-1 in skeletal muscle enhances insulin sensitivity, glucose tolerance and energy homoeostasis.

463

HYPOXIA-INDUCIBLE FACTOR-1A GENE POLYMORPHISMS AND THE RISK OF PREECLAMPSIA IN A SINHALESE POPULATION FROM SRI-LANKA

P. H. Andraweera[1], S. D. Thompson[1], V. H.W. Dissanayake[2], R. W. Jayasekara[2], G. A. Dekker[1,3], C. T. Roberts[1]

[1]Discipline of Obstetrics and Gynaecology,Research Centre for Reproductive Health, University of Adelaide, Adelaide, SA, Australia

[2]Human Genetics Unit, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka

[3]Women's and Children's Division, Lyell McEwin Hospital, Elizabeth Vale, SA, Australia

Introduction: Hypoxia-Inducible-Factor-1α (HIF-1α) regulates the expression of several genes involved in placental angiogenesis, such as vascular endothelial growth factor (VEGF) and VEGF receptor-1 (FLT1). Maternal plasma VEGF and placental expression of VEGF are reduced in preeclamptic (PE) pregnancies. We aimed to determine whether single nucleotide polymorphisms in the HIF-1α gene (HIF-1α rs11549465 and rs10873142) are associated with preeclampsia.

Methods: 180 nulliparous Sinhalese women with preeclampsia and 180 normotensive women matched for age, ethnicity, parity and BMI were recruited from two tertiary care hospitals in Colombo. Women with a BMI ≥ 30 kg/m2 were excluded. Preeclampsia was diagnosed using international guidelines. Early preeclampsia (n=73) was defined as onset of preeclampsia prior to 34 weeks of gestation. A small for gestational age baby was defined as having a birthweight below the 10th customised birthweight centile (n=102). Peripheral blood was collected from the participants and DNA extracted. Genotyping was performed using Sequenom MassARRAY. Genotype and allele frequencies of cases were compared with controls using chi square.

Results: HIF-1α rs11549465 CC genotype [P=0.002, OR(95% CI)=3.3(1.5-7.5)] and C allele [P=0.003, OR(95% CI)=3.1(1.4-6.7] were increased in women who developed early onset preeclampsia compared to controls and to those who developed PE after 34 weeks gestation [P=0.005, OR(95% CI)=3.3(1.4-7.7)] and [P=0.006, OR(95% CI)=3.0(1.3-6.8)]. The CC genotype [P=0.017, OR(95% CI)=2.2(1.1-4.4)]

and C allele [P=0.02, OR(95% CI)=2.1(1.1-4.0)] were also increased in preeclamptic pregnancies resulting in the delivery of a small for gestational age baby. HIF-1α rs10873142 was not associated with pregnancy outcome.

Conclusion: HIF-1α rs11549465 C allele has previously been shown to have lower transcriptional activity and to reduce angiogenesis. Our results demonstrate the association of this allele with early PE and PE+SGA. Inherited susceptibility to low HIF-1α expression resulting in reduced expression of angiogenesis regulating genes may be implicated in the pathophysiology.

464

DIFFERENTIAL EXPRESSION OF MICRORNA WITH LABOUR IN THE HUMAN MYOMETRIUM

E. Chan, R. Smith

Endocrine Unit, Mothers and Babies Research Centre, Hunter Medical Research Institute, The Unive, New Lambton Hts, Australia

Normal human birth requires the activation of physiological systems that lead to coordinated uterine contractions and full cervical dilatation. We have data that show ERα expression in the term human myometrium increases from not-in-labour to in-labour; and that ERα mRNA is highly correlated with mRNA changes for the proteins that mediate myometrial contractility: connexin 43 (Cx43), cyclo-oxygenase 2 (Cox2) and NFκB. There is also considerable published evidence linking microRNA with estrogen action, the regulation of Cx43, NFκB, and Cox2 in various tissues and myocyte contractility, however, this is yet to be explored in human myometrium.

This study aims to ascertain whether miRNA are differentially expressed with labour in the term human myometrium.

We performed miRNA expression profiling of 377 unique miRNA species using the Taqman® miR Array with 384-well cards (TLDA Plate A, Sanger version 10, Applied Biosystems). Total RNA extracts of myometrial tissues from 4 subjects at term before labor onset and 4 subjects in whom labour had begun were reverse-transcribed using Taqman® MegaPlex Pool A primers and real-time PCR performed. Among the 174 detectable miRNAs in our 8 samples, 8 miRNA species were significantly altered by labour ($P<0.01$ for both increased and repressed miRNA expression. Most miRNAs were elevated in the labouring compared to the non-labouring tissues. Of particular interest is miRNA-93, which has a predicted target site on αB-crystallin (MicroCosm Targets Version 5). An increased expression of miRNA-93 with labour may inhibit the translation of αB-crystallin mRNA producing the significant 3.3-fold decrease with labour that we found in our previous 2-D DIGE proteomic study. In addition several of the miRNAs consistently decreased in the labouring tissues are predicted to target ERα and the CAPs, including miR-133a, which has a target site on NFκB p65 subunit.

These results support a potential role for miRNA in human labour.

465

MATERNAL PLASMA CIRCULATING LEVELS OF OMEGA (N)3 LONG CHAIN POLYUNSATURATED FATTY ACIDS, EICOSAPENTAENOIC ACID (EPA), DOCOSAPENTAENOIC ACID (DPA) AND DOCOSAHEXAENOIC ACID (DHA) ARE ASSOCIATED WITH FETAL GROWTH MEASURES

P. C. McLernon[1], V. E. Murphy[2], L. G. Wood[2], G. A. Dekker[1], N. Hodyl[1]V. L. Clifton[1]

[1]Research Centre for Early Origins of Health and Disease - Pregnancy and Developm, The Robinson Institute, Lyell McEwin Hospital, Elizabeth Vale, SA, Australia

[2]Department of Respiratory and Sleep Medicine, Hunter Medical Research Institute, Newcastle, NSW, Australia

Introduction: Although pregnancies complicated by asthma have been associated with reduced fetal growth, omega (n)3 fatty acids may confer a protective effect against the inflammatory response and subsequently mediate fetal growth. We examined the relationship between circulating levels and dietary intake of n3s in pregnancies complicated by asthma and related these to fetal growth.

Methods: Peripheral blood was collected from, and a 24 hour food recall questionnaire was completed by asthmatic (n=85) and non-asthmatic (n=50) pregnant women at gestational weeks (G) 18, 30 and 36. Plasma circulating n3s were analysed using gas chromatography and dietary analysis was conducted using Foodworks software. Fetal growth parameters were analysed by ultrasound at each gestational visit. Birth weight centile (BWC) groups were dichotomously categorised into low (<26) and other (≥26).

Results: Circulating EPA and DPA (but not DHA) were significantly lower in the low (BWC) group than the high BWC group (ANOVA; $p<.05$) at G18. There was no consistent pattern of circulating EPA over pregnancy in the low BWC group for either asthmatics or controls. Circulating EPA was systematically reduced over pregnancy in the high BWC group for both the asthmatics and controls. There were no significant differences between maternal dietary intake of EPA, DPA or DHA, nor of maternal energy intake.

In the asthmatics, maternal circulating levels of DHA at G18, 30 and 36 in the low BWC group were negatively correlated with head circumference (HC) and biparietal diameter (BPD) at G36 ($p<.05$). Irrespective of BWC category, in the asthmatic group, maternal circulating levels of DHA at G18, 30 and 36, as well as EPA and DPA at G36 were negatively correlated with head circumference (HC) at G36 ($p<.05$). Interestingly, this pattern of results was not found in the control group.

Conclusion: Under normal developmental conditions, the fetus seems able to utilise the n3 fatty acids available over the course of pregnancy. It appears that placental uptake and preferential transfer of these n3fatty acids is impaired in fetuses on a low BWC developmental trajectory. Further, these data suggest that if fetal ontogeny develops under asthmatic conditions, it may be either asthma or its treatment which hinders the capacity of the fetoplacental unit to utilise n3s in order to enhance fetal growth. Under such conditions, maternal supplementation with DHA may not improve fetal growth.

466

CIRCULATING GROWTH HORMONE PROFILES REMAIN PULSATILE DURING PREGNANCY IN PIGS

K. L. Gatford[1], K. Taylor[1,2], K. L. Kind[2], W. H.E. Van Wettere[2], J. A. Owens[1]

[1]School of Paediatrics and Reproductive Health and Robinson Institute, University of Adelaide, University of Adelaide, SA, Australia

[2]School of Animal and Veterinary Sciences, University of Adelaide, Roseworthy, SA, Australia

Growth hormone (GH) plays an important role in maternal adaptation to pregnancy. Maternal levels of GH are low in human pregnancies with poor fetal growth (1), and administration of exogenous GH increases fetal growth and placental function in non-human species including pig and sheep (2, 3). In growing animals, responses to GH depend on the pattern of delivery as well as the dose (4). Little is known about how circulating GH profiles change during pregnancy in non-human species, and which characteristics of maternal GH profiles affect fetal growth. We therefore characterised circulating GH profiles before and during pregnancy in the young pig, in which fetal growth is constrained commercially by restricted maternal feeding as well as continued maternal growth.

Blood samples (10 minute intervals, 6 h) were collected serially in young female pigs before their first mating (n=5), and early (30 d after mating, n=10), mid (d 65, n=6) and late (d 100, n=7) in their first pregnancies (term ~115 d). Where possible, the same pigs were sampled at multiple stages. Litter size and piglet birth weights were recorded at delivery. Plasma GH levels were measured by RIA and pulse characteristics fitted by Pulsar. Effects of pregnancy were analysed by 1-way ANOVA, and relationships with litter outcomes were analysed by Pearson's correlation. Results are mean ± SEM.

Mean plasma GH fluctuated through pregnancy ($P = 0.044$), being highest before mating and at mid-pregnancy, and remained pulsatile, with similar GH pulse frequencies and amplitudes before and throughout pregnancy ($P>0.1$ for each). Measures of pulsatile, but not basal, maternal circulating GH during late pregnancy correlated positively with litter birth weights.

Endogenous pulsatile circulating GH in the pregnant pig positively predicts birth weight of her progeny. This suggests that strategies to increase endogenous maternal GH may promote fetal growth, even under conditions that restrict fetal growth.

(1) HD McIntyre, R Serek, et al (2000) JCEM 85:1143-1150
(2) KL Gatford, MJ De Blasio, et al (2009) J Endocrinol 203:143-154
(3) JE Harding, PC Evans and PD Gluckman (1997) Endocrinology 138:5352-5358
(4) RG Clark, J-O Jansson, et al (1985) J Endocrinol 104:53-61

467

THE INFLAMMATORY STATE OF THE RAT PLACENTA INCREASES AT TERM AND IS FURTHER ELEVATED BY EXPOSURE TO DEXAMETHASONE

J. L. Lewis[1], P. J. Mark[1], M. L. Jones[1], J. A. Keelan[2], B. J. Waddell[1]

[1]School of Anatomy & Human Biology, The University of Western Australia, Nedlands, WA, Australia

[2]School of Women's and Infants' Health, The University of Western Australia, Subiaco, WA, Australia

Successful reproduction is dependent upon inflammation, with ovulation, implantation and parturition all involving inflammatory processes. Dysregulation of inflammation, however, is linked to a number of pregnancy pathologies including preeclampsia, intrauterine growth restriction and pre-term labour. Glucocorticoids are well recognised for their potent anti-inflammatory effects in virtually all tissues, where they suppress expression of pro-inflammatory cytokines including tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6. Glucocorticoids also promote fetal maturation and limit placental growth, but whether they exert anti-inflammatory effects in placenta is unknown. The aim of this study was to characterise the inflammatory state of the rat placenta in late gestation with and without maternal glucocorticoid treatment. Placentas (n=6/group) from control (Con) and dexamethasone-treated rat pregnancies (Dex; 0.75 μg/ml drinking water from day 13 of gestation) were collected at days 16 and 22 (term = 23 days). Placentas were dissected into junctional (JZ) and labyrinth (LZ) zones for separate analysis. Quantitative PCR was used to determine placental expression of mRNA for pro-inflammatory cytokines and inflammation modulators including TNF-α, IL-1β, IL-6 and the cyclooxygenase/prostaglandin G/H synthase isoforms (Ptgs-1 and -2). JZ and LZ expression of TNF-α, IL-1β and IL-6 mRNA increased 2- to 4-fold between days 16 and 22, whereas Ptgs-2 increased only in the LZ. Ptgs-1 was unaffected by gestational age in either zone. Surprisingly, Dex treatment did not suppress cytokine expression in either zone on both days. On the contrary, Dex increased LZ expression of IL-1β (2-fold) on day 16 , and increased JZ expression of Ptgs-1 on both days and expression of Ptgs-2 in both zones on day 22. These data clearly demonstrate that the inflammatory state of the placenta increases near term and, in contrast to other tissues, glucocorticoids appear to exert pro-inflammatory effects in the placenta.

468

GPR30, THE NOVEL MEMBRANE ESTROGEN RECEPTOR, STIMULATES CONTRACTILITY OF MYOMETRIUM BY INCREASING EXPRESSION OF H-CALDESMON.

K. Maiti, J. W. Paul, M. Read, E. C. Chan, R. Smith

endocrinology, MBRC, John Hunter Hospital, new lambton, NSW, Australia

Context: Recently, a novel cell surface receptor for estrogen, called GPR30, was identified in cancer cells. Our real time PCR data and western blot data showed that both GPR30 mRNA and protein are expressed in human pregnant myometrium and that GPR30 can induce

rapid non-genomic signalling in myometrial explants culture through phosphorylation of mitogen activated protein kinase (MAPK) and HSP27. Additionally, G1, a GPR30 agonist and estradiol increased contractility of myometrium in response to oxytocin.

Objective: We investigated the cellular localization of GPR30 in myometrium from pregnant women at term and the signalling pathways activated by this receptor for stimulation of myometrial contractility.

Methods: Myometrial tissues were collected from elective or emergency caesarean sections. We performed fluorescence immunohistochemistry for GPR30 in paraffin embedded myometrial tissue sections. Additionally, we treated the myometrial tissue explants for 12hrs with estradiol and G1, GPR30 specific agonist. Myometrial explants culture was used to check the expression of h-caldesmon after treatment with G1 or estradiol.

Results: The confocal immunofluorescence data showed that GPR30 is localised in the plasma membrane and caveolae in non-labouring and labouring myometrial cells. GPR30 is co-localized with caveolin-1 in both labouring and non-labouring myometrium. Furthermore, GPR30 agonist, G1 and estradiol increased the expression of caldesmon within 12 hrs in myometrial tissue culture.

Conclusion: The interaction between GPR30 and caveolin-1 in myometrium, may prove to be essential for controlling myometrial function, for instance in receptor desensitization or trafficking. The increased myometrial contractility caused by G1 and estradiol, may be due to increased expression of h-caldesmon which is involved in actin filament formation and actin-myosin interaction.

469

CPG ISLAND METHYLATION OF PROINFLAMMATORY AND STEROID RECEPTOR GENE PROMOTERS IN THE HUMAN AMNION

C. M. Mitchell[1,3], A. M. Bisits[1,2,3], S. D. Sykes[1,4], K. G. Pringle[1,4], E. R. Lumbers[1,4], T. Zakar[1,2,3]

[1]Mothers and Babies Research Centre, HMRI, Newcastle, NSW, Australia
[2]Obstetrics and Gynaecology, John Hunter Hospital, Newcastle, NSW, Australia
[3]School of Medical Practice and Public Health, University of Newcastle, NSW, Australia
[4]School of Biomedical Sciences, University of Newcastle, NSW, Australia

CpG island methylation in promoters is associated with the repression of gene activity. To explore the involvement of this epigenetic modification in controlling gene expression in the amnion, we have determined the methylation density of CpG islands in the proinflammatory genes *PTGS2*(prostaglandin synthase-2), *BMP2*(bone morphogenetic protein-2), *NAMPT*(pre-B-cell colony stimulating factor), and *CXCL2*(chemokine-CXC-motif ligand-2), which are induced in the fetal membranes in late pregnancy and with labour, of *NR3C-1* (glucocorticoid receptor), which is constitutively expressed and of *PGR* (progesterone receptor) and *ESR1* (estrogen receptor-α) genes, which are repressed throughout gestation. Amnion was collected at 10.8-17.8 weeks of pregnancy, at term and after term labour (n=8, each) and analysed using a customised Methyl-Profiler assay (SABiosciences), which quantified the percentage of highly methylated, unmethylated and intermediately methylated CpG islands.

CpG islands were either highly methylated or unmethylated, but no CpG island was intermediately methylated. The proportion of highly methylated CpG islands varied widely between samples, but median values were low (Table) and not different between patient groups for any gene (Kruskal-Wallis ANOVA).

Percent of Highly Methylated CpG islands

Gene	*PTGS2*	*BMP2*	*NAMP T*	*CXCL2*	*NR3C1*	*PGR*	*ESR1*
Median	0.24	1.06	0.42	2.58	0.99	0.44	11.50
Max	13.56	39.87	30.17	42.32	28.58	26.18	50.93
Min	0.00	0.24	0.01	0.32	0.25	0.03	6.91
	N= 23	N= 20	N= 23	N= 22	N= 24	N= 24	N= 22

ESR1 methylation was higher than all other genes, while *PTGS2* methylation was lower than *BMP2*, *CXCL2* and *NR3C-1*. *NAMPT* and *CXCL2* methylation also differed significantly (Kruskal-Wallis, Bonferroni multiple comparisons). Thus CpG island methylation in amnion is established before 10 weeks of pregnancy in a gene specific fashion and maintained until term. Repression of gene activity via this epigenetic signal may be substantial in a subgroup of individuals; however, additional transcriptional and epigenetic mechanisms may control the overall expression of these genes during pregnancy and labour.

470

IS SEASONAL VARIATION IN VITAMIN D DEFICIENCY DURING PREGNANCY REFLECTED BY SEASONAL VARIATION IN PREECLAMPSIA AND GESTATIONAL DIABETES MELLITUS?

G. S.Y. Ong[1], A. E. Makepeace[1], J. Ng[1], S. Brown[1], N. Hadlow[2,3], E. Lim[1,3]

[1]Department of Endocrinology and Diabetes, Sir Charles Gairdner Hospital, Nedlands, WA, Australia
[2]Western Diagnostic Pathology (Symbion), Myaree, WA, Australia
[3]PathWest Laboratory Medicine, Nedlands, WA, Australia

Introduction: Vitamin D deficiency in pregnancy has adverse consequences, such as neonatal hypocalcaemic convulsions, rickets in infancy and reduced bone mass in childhood. Furthermore, maternal vitamin D deficiency may predispose to preeclampsia (PE) and gestational diabetes mellitus (GDM). We report the prevalence of vitamin D deficiency in pregnant women receiving routine antenatal care, as well as the incidence of PE and GDM in Western Australia.

Method: Vitamin D was measured on serum obtained from 485 consecutive women attending Western Diagnostic Pathology for first trimester screening during February 2008 [summer] and 496 women during August 2008 [winter]. Vitamin D was analysed by chemiluminescent immunoassay [Diasorin Liaison]. Incidence of PE and GDM were captured from the Department of Health Western Australia midwife registry. Assessment for seasonal variation in these conditions utilised the date of delivery as the reference time point.

Results:

	Summer	Winter
Number	485	496
Mean Age	31	31
Mean Gestational Weeks	11.3 ±1.1	11.2 ±1.0
Vit D <25 nmol/L	5 (1%)	51 (10%)
Vit D 25-50 nmol/L	66 (14%)	222 (45%)
Vit D 50-80 nmol/L	265 (55%)	208 (53%)
Vit D > 80 nmol/L	148 (31%)	15 (3%)

Season of Delivery	Summer	Autumn	Winter	Spring
PE	213/7291(3%)	213/7538(3%)	206/7417(3%)	235/7250(3%)
GDM	347/7157(5%)	336/7415(5%)	353/7270(5%)	338/7147(5%)

The mean vitamin D levels in summer and winter were statistically different ($p<0.001$). There was no correlation between maternal age and vitamin D level. There was no statistically significant difference in the incidence of PE ($p=0.37$) or GDM ($p=0.80$) between seasons of date of delivery.

Conclusions: Moderately severe deficiency [<25 nmol/L] was seen in 1% during summer, increasing to 10% in winter. The lack of statistically significant differences in the seasonal incidence of PE or GDM suggests that seasonal variation in Vitamin D does not influence susceptibility to PE or GDM.

471

CALDESMON PHOSPHORYLATION AND PHASIC REGULATION OF ERK 1/2 DURING CONTRACTIONS IN HUMAN MYOMETRIUM

J. Paul, K. Maiti, M. Read, R. Smith

Mothers and Babies Research Center, University of Newcastle, Newcastle, NSW, Australia

BACKGROUND: Within human myometrium the actin binding protein caldesmon (h-CaD) inhibits myosin ATPase activity, preventing the generation of contractile force. Upon phosphorylation, h-CaD undergoes a conformational change that relieves myosin ATPase inhibition as well as expose the actin filament groove. These changes promote actin-myosin cross bridge cycling and the generation of contractile force. Evidence suggests h-CaD phosphorylation is mediated by ERK 1/2. Here we reiterate h-CaD phosphorylation during contractions and demonstrate importance of the contraction phase in capturing evidence of ERK 1/2 regulation. HYPOTHESIS/AIMS: We hypothesised h-CaD regulates development of tension in human myometrium during onset of contractions, and that factors regulating h-CaD phosphorylation would themselves exhibit phasic regulation in parallel to contractions. We aimed to test this hypothesis through examining h-CaD phosphorylation in non-labouring (NL) and labouring (L) human myometrium, as well as tissue frozen at peak contraction and relaxation following the onset of spontaneous contractions *in vitro.* METHODS: Term NL (n=8) and L (n=8) myometrial samples were collected, washed and snap frozen for protein analysis. Fresh NL tissue (n=5) was also dissected into strips and suspended in organ baths under 1 g tension. Transducers were used to observe the onset of spontaneous contractions. Strips were snap frozen at specific contraction phases, including; (i) prior to the onset of any contractions, (ii) maximum contraction, and (iii) relaxed between contractions. Proteins were extracted, separated using SDS-PAGE and western transferred. Phospho-specific antibodies were then used to detect target proteins.

RESULTS: The onset of labour *in vivo* (NL vs L) was associated with a 2-fold increase in h-CaD phosphorylation ($p=0.012$) relative to unchanged total h-CaD expression. Similarly, during *in vitro* studies h-CaD phosphorylation was 2-fold higher ($p=0.04$) in tissue frozen at peak contraction (point ii) compared to tissue frozen prior to the onset of contractions (point i). Interestingly, analysis of ERK 1/2 phosphorylation revealed no change when comparing NL and L samples, however analysis of *in vitro* samples revealed ERK 1/2 was phasically phosphorylated and de-phosphorylated in parallel to contractions.

DISCUSSION: NL and L myometrial samples are routinely compared to highlight changes associated the onset of labour. Our results demonstrate that whilst many changes can be observed using this approach, such as h-CaD phosphorylation, other changes are highly transient, such as ERK 1/2 phosphorylation, and may only be observed at specific stages of contractions.

472

THE EFFECT OF ANTHELMINTICS ON OVARIAN FUNCTION IN MERINO EWES

S. E. Plug, P. A.R. Hawken, G. B. Martin

Institute of Agriculture, University of Western Australia, Crawley, WA, Australia

Anthelmintics are routinely used in sheep enterprises for the control of gastrointestinal worms. The time of mating relative to drench administration is not currently considered a management issue, but recent studies suggest that anthelmintics from the benzimidazole and imidazothiazole classes may interfere with steroidogenesis because they reduce the concentration of oestradiol in follicular fluid and progesterone in the female reproductive tract. These hormones control oocyte growth and ovulation rate, and changes in the delicate balance of these hormones could have large impacts on fertility and reproductive performance. In this study we tested whether ewes treated with anthelmintics would have lower concentrations of follicular oestradiol and plasma progesterone, higher ovulation rate, and delayed ovulation compared with untreated ewes. Groups of 30 adult, merino ewes received three injections of prostaglandin to synchronise ovulation. At the time of the last injection, ewes were either drenched with albendazole, a fenbendazole/levamisole mix or water (control) and the time of mating, ovulation rate and concentration of plasma progesterone were measured. A subset of ewes (n = 5 / group) were killed to assess follicle number and the concentration of follicular oestradiol. Ewes treated with albendazole had 49% less twin ovulations than the control ewes ($P < 0.01$) and ewes receiving the mixed drench had 25% less twin ovulations than the control ewes but this divergence failed to reach significance ($P = 0.09$). This effect was reflected by a lower ovulation rate in the albendazole-treated group (1.3 ± 0.1) and the mixed drench group (1.5± 0.1), compared to the control group (1.8 ± 0.1). No change was found in the concentrations of oestradiol or progesterone ($P > 0.50$), or time of ovulation ($P > 0.50$). These changes in ovulation rate may impact on the fertility and reproductive performance of ewes, potentially lowering farm productivity.

473

DECREASED UTERINE PROGESTERONE RECEPTOR EXPRESSION MAY EXPLAIN FUNCTIONAL PROGESTERONE WITHDRAWAL IN THE PREGNANT GUINEA PIG

T. Welsh[1], S. Mesiano[2], J. Hirst[1], T. Zakar[1]

[1]Mothers and Babies Research Centre, University of Newcastle, Newcastle, NSW, Australia

[2]Department of Reproductive Biology, Case Western Reserve University, Cleveland, OH, United States

Parturition occurs in the presence of high circulating progesterone levels and exogenous progestin administration does not prolong gestation in the guinea pig. Labour can be induced, however, by early withdrawal of progesterone (by the use of progesterone receptor (PR) antagonists or ovariectomy in early pregnancy). It is hypothesised that the progesterone responsiveness of the guinea pig myometrium decreases at term, causing "functional" progesterone withdrawal prior to normal labour onset. We examined the expression of PRs in the pregnant guinea pig uterus from 45 days (~2/3 of gestation) through to term (~68 days) to determine if altered PR expression may account for decreased progesterone responsiveness at term labour. There was a significant decrease in uterine PR-A and -B protein content with advancing gestation and labour onset; PR-A and –B levels were decreased by 87% and 86%, respectively, at labour compared to 45d. We did not detect expression of alternative PR isoforms, such as the putative PR-C. Estrogen receptor (ER)-α protein levels were also measured and did not change with gestation or labour. Administration of the metabolism-resistant PGE_2 analogue Sulprostone (0.25 mg single dose) at 45 days gestation significantly decreased uterine PR-A levels ($P = 0.0168$) within 16 hours and caused a trend towards lower PR-B levels, with no change in ERα levels. In single horn pregnancies, we found significantly lower PR-A ($P = 0.0026$), PR-B ($P = 0.00009$) and ERα ($P = 0.0026$) levels in myo-endometrium from gravid compared to non-gravid horns, suggesting that PR and ERα expression in the pregnant uterus is suppressed by stretch and/or local paracrine factors. These data indicate that functional progesterone withdrawal in the pregnant guinea pig is mediated by decreased uterine expression of PR-A and –B and may be induced or augmented by prostaglandins *in vivo*.

474

DIFFERENTIAL EXPRESSION OF HEPATIC MICRORNAS IN PRENATALLY FOLIC ACID SUPPLEMENTED NEONATES

W. H. Chu[2], M. L. Harland[1], P. A. Grant[2], M. J. De Blasio[2], W. C. Kong[2], S. Moretta[2], J. S. Robinson[2], M. E. Dziadek[3], J. A. Owens[2]

[1]Obstetrics and Gyanecology, University of Adelaide, Adelaide, SA, Australia
[2]Robinson Institute, Adelaide, SA, Australia
[3]Garvan Research Institute, Sydney, NSW, Australia

Antenatal folic acid supplementation is recommended to reduce the risk of neural tube defects in the newborn. As a methyl donor, folic acid participates in DNA and histone methylation, both major epigenetic processes *in utero* and may therefore alter the epigenome, gene expression and cellular function. We have previously shown that antenatal maternal folic acid supplementation (MFAS) alters hepatic expression of microRNAs and functional genes related to metabolism and insulin action in adult progeny. The aim of the current study was to determine if this was established at birth by examining the effect of MFAS on hepatic microRNA expression in neonatal progeny.

Female Wistar rats were fed one of two diets: Control (n=8, 2mg folic acid/kg) or Folic Acid Supplemented (n=8, 6mg folic acid/kg), from two weeks before mating and throughout pregnancy. One male and female progeny per litter were killed on postnatal day 1. Neonatal hepatic microRNA expression was determined by Exiqon microRNA microarray v.11. Predicted targets of differentially expressed microRNAs and their molecular networks were identified.

MFAS altered expression of eighteen hepatic microRNAs ($p<0.05$) in the neonatal male offspring, with 5 down regulated and 13 upregulated, including miR-122a, which increases lipogenesis and decreases fatty acid oxidation. MFAS also altered expression of eleven hepatic microRNAs ($p<0.05$) in the neonatal female offspring, with 4 downregulated and 7 upregulated. Other functions highly associated with the predicted targets of these differentially expressed microRNAs include cellular development, cellular assembly and organisation.

MFAS alters the expression of hepatic microRNAs in offspring in early postnatal life, with upregulation of hepatic miR-122a in males, which possibly leads to excess lipid accumulation during lactation.

475

PRETERM BIRTH AND INTRAUTERINE GROWTH RESTRICTION: EFFECT ON MICROVASCULAR FUNCTION IN THE NEONATAL GUINEA PIG

R. M. Dyson[1,2], H. K. Palliser[1,3], M. A. Kelleher[1,3], J. J. Hirst[1,3], I. M.R. Wright[1,2,4]

[1]Mothers & Babies Research Centre, Hunter Medical Research Institute, Newcastle, NSW, Australia
[2]School of Medicine & Public Health, University of Newcastle, Newcastle, NSW, Australia
[3]School of Biomedical Sciences & Pharmacy, University of Newcastle, Newcastle, NSW, Australia
[4]Kaleidoscope Neonatal Intensive Care Unit, John Hunter Children's Hospital, Newcastle, NSW, Australia

Microvascular dysfunction, characterised by inappropriate vasodilatation and high blood flow throughout the peripheral microcirculation, has been linked to levels of physiological instability and poor outcome in the newborn neonate. Specifically, preterm neonates have significantly higher levels of baseline microvascular flow than term neonates at 24 hours postnatal age. In humans, intrauterine growth restriction (IUGR) is not known to affect microvascular function in early extrauterine life. Previous work shows microvascular blood flow is comparable in Small for Gestational Age (SGA) and non-SGA infants at 24 hours postnatal age. To better understand the mechanisms underlying microvascular function in at-risk groups during this period, we have established an animal model of early extrauterine life using the neonatal guinea pig. Preterm delivery at 63±1 days gestation (full term= 70 days) of guinea pig neonates results in considerable immaturity and decreased viability (54% mortality compared to 9% in term controls). IUGR (characterised by post-mortem brain-to-liver weight ratio) occurs spontaneously in this species. Laser Doppler flowmetry (LDF) was used to study microvascular blood flow at 23 hours postnatal age in surviving preterm (n=33) and term (n=25) animals. LDF analysis showed baseline microvascular flow was significantly higher in preterm (1.21 (0.84-1.67) logPU) than term (1.0 (0.63-1.36) logPU) animals (p=0.0009). No effect of IUGR on baseline flow was observed in either preterm (n=11 IUGR, n=22 non-IUGR; p=0.7435) or term (n=9 IUGR, n=16 non-IUGR; p=0.9724) animals. These results are in line with recent clinical findings and therefore further support the guinea pig as a suitable model for future studies of the mechanisms underlying microvascular behaviour in the initial extrauterine period.

476

IUGR ALTERS RNO-MIR-16, -18A AND -142-3P IN DAY 260 OMENTAL FAT BUVL RAT OFFSPRING TISSUE

H. Harryanto, B. L. Summers-Pearce, M. J. De Blasio, S. Moretta, T. A. How, P. A. Grant, J. A. Owens

Discipline of Obstetrics and Gynaecology, Research Centre for Early Origins of Health & Disease, Robinson Institute, Unive, Adelaide, SA, Australia

Introduction: Placental restriction (PR) is a major cause of intrauterine growth restriction (IUGR), which is associated with adult onset type 2 diabetes. This appears mediated through down-regulation of gene and/or protein expression of insulin signalling pathway in adipose tissue (1). MicroRNAs (miRNAs) are small non-protein coding RNA that can down-regulate expression of multiple target mRNAs and/or proteins. We hypothesised that PR alters of miRNAs expression in adipocytes of progeny, some may target the insulin signalling pathway.

Method: Placental restriction in the pregnant rat induced by bi-artery vein ligation (BUVL) of the placenta at day 18 of pregnant dams (2). Omental fat was collected during post-mortem at day 260. Total RNA was extracted by TRIzol and miRNAs expression were analysed by Exiqon miRCURY arrays v.11.0, then verified by qRT-PCR. Predicted targets were identified by miRecords database then subjected to Ingenuity Pathway Analysis (IPA) to identify any targeted networks.

Results: BUVL reduced body weight offspring from post-natal day 3 to 40 ($P < 0.05$) in PR offspring. At day 260, catch-up growth only observed in male and a brain sparing condition observed in female PR offspring. PR increased expression of rno-miR-16, -18a and -142-3p in omental fat of day 260 BUVL rat offspring (fold change 1.38 – 2.40, adjusted *P*-value < 0.05). These miRNAs were predicted to target molecules involved in amino acid, lipid and carbohydrate metabolism in adipose tissues. Two of them are PCK1(rno-miR-18a) which is a main control point for gluconeogenesis and PDE3B (rno-miR-16) which regulates insulin receptor expression by protein-protein interaction and a negative regulator of lipid catabolic process. Rno-miR-16 and -18a expression could epigenetically altered by DNA methylation as indicated by the presence of CpG islands.

Conclusion: Early perturbation by PR able to alter miRNAs expression in omental fat tissue of rat offspring and potentially may alter metabolic pathways.

(1) Jaquet D et al. (2001) J Clin Endocrinol Metab 86(7): 3266-3271
(2) Wigglesworth JS (1974) Am J Pathol 77(2): 347-350

477

PREMATURE BIRTH RESULTS IN *EX UTERO* BRAIN DEVELOPMENT IN A LOW NEUROPROTECTIVE STEROID ENVIRONMENT.

M. A. Kelleher[1], H. K. Palliser[1], J. J. Hirst[2]

[1]Mothers & Babies Research Centre, University of Newcastle, Newcastle, NSW, Australia
[2]School of Biomedical Sciences, University of Newcastle, Newcastle, NSW, Australia

Preterm infants are at a higher risk of developing neurological, motor and cognitive disorders resulting in long-term morbidity. Allopregnanolone, a neuroactive metabolite of progesterone and potent modulator of neural excitability, has neuroprotective properties in the fetal and neonatal brain. Concentrations of progesterone and allopregnanolone fall dramatically after birth. We hypothesise that preterm infants are prematurely exposed to low neurosteroid levels, adversely affecting normal brain development and neuroprotection. We have developed a preterm neonatal model in the guinea pig in which neonates are delivered by c-section at 62-63 days of gestation or at 69 days (term=70 days). Animals received a single course of betamethasone 24hrs prior to delivery and CPAP (continuous positive airway pressure) and surfactant administration immediately following birth. Neonates are housed in a humidified incubator and receive regular assisted feeding. At 24hrs post-delivery animals were euthanased and brains were collected and processed for radioimmunoassay of allopregnanolone, immunoblotting (region containing hippocampus, cortex and thalamus) of 5 a R1 & 5 a R2 and immunohistochemical analyses of MBP (Myelin Basic Protein) and GFAP (Glial Fibrillary Acidic Protein) in the hippocampal CA1 region and sub-cortical white matter. Brain tissue from fetal (GA63) and term fetal (GA69) animals was collected for comparison. At 24hrs old, preterm guinea pigs were developmentally immature when compared to term neonates with MBP & GFAP expression in the CA1 and sub-cortical white matter reduced by ~30% ($P<0.05$). The 24hr old preterm neonates also had ~60% less brain allopregnanolone than concentrations measured in fetal brains at term (4.0 ± 0.6 ng/g vs 10.2 ± 0.9 ng/g, respectively). Preterm neonatal brain expression of the neurosteroid synthetic enzyme, 5 a R2, was also significantly lower than the expression in term brains ($P<0.05$). These premature guinea pig neonates face the challenge of "catch-up" neurodevelopment & maturation in the absence of the high levels of allopregnanolone that are present *in utero* during late gestation growth. They also have a reduced capacity to synthesise allopregnanolone than at term, potentially leaving them more vulnerable to subsequent insult, poor neurodevelopmental outcomes and long-term disability.

478

MATERNAL FOLIC ACID SUPPLEMENTATION IN THE RAT ALTERS PANCREATIC GENE EXPRESSION IN ADULT PROGENY.

S. N. Mohammad[1], W. Kong[1], P. A. Grant[1], S. Moretta[1], M. J. De Blasio[1], L. Harland[1], K. L. Gatford[1], J. S. Robinson[1], M. Dziadek[2], J. A. Owens[1]

[1]Research Centre for Early Origins of Adult Disease, Robinson Institute, The University of Adelaide, Adelaide, SA, Australia
[2]Garvan Institute for Medical Research, Darlington, NSW, Australia

Background: Exposure of pregnant women to high folic acid intake is common and likely to increase. In humans, increased maternal plasma folate is predictive of insulin resistance in progeny, but whether insulin secretion in also affected has not been studied. We have recently shown that maternal folic acid supplementation (MFAS) in the rat improves stimulated insulin secretion in adult male, but impairs this in females. To better understand the mechanisms underlying these functional changes, we investigated the effects of MFAS in the rat on β-cell mass and pancreatic expression of key regulatory and functional genes in adult progeny.

Methods: Pregnant rats were fed a control (2mg folic acid/kg) or FAS (6mg/kg) diet from two weeks before and throughout pregnancy. Pancreas was collected from male and female control and FAS progeny at 3 mo of age following measurement of in vivo insulin secretion. β-cells and islets were counted after immunohistochemical staining for insulin. Gene expression was measured by GeXP and expressed relative to mitochondrial ribosomal protein L19 (mrpl19).

Results: MFAS did not alter β-cell mass, the proportion of small islets or islet density in progeny. MFAS reduced pancreatic expression of Glucagon-like-peptide-1 receptor (GlpR, P=0.045) and more so in female than male progeny (P=0.055). MFAS tended to reduce pancreatic expression of insulin1 and insulin2 (P=0.067, P=0.087 respectively) and when adjusted for β-cell mass (P=0.039, P=0.071 respectively) in progeny. MFAS also tended to reduce $K_{ir}6.2$ (major subunit of the ATP-sensitive K+ channel, an inward-rectifier potassium ion channel, KCNJ11) in females only (P=0.087).

Conclusion: MFAS alters pancreatic expression of key regulatory and functional genes in progeny and to a greater extent in females, consistent with their impaired insulin secretion.

479

THE IMPACT OF PREGNANCY AND LACTATION ON BONE IN RAT MOTHERS EXPOSED TO UTEROPLACENTAL INSUFFICIENCY

M. E. Wlodek[1], J. D. Wark[2], T. Romano[1,2]

[1]Physiology, The University of Melbourne, Parkville, VIC, Australia
[2]Medicine, Department of Medicine, The University of Melbourne, Bone and Mineral Service, R, Parkville, VIC, Australia

Pregnancy and lactation effect maternal bone mass providing offspring with calcium requirements. Uteroplacental insufficiency complicates 10% of human pregnancies causing intrauterine growth restriction, lower pup body calcium and programming of bone deficits. We determined whether mothers exposed to uteroplacental insufficiency have altered skeletal phenotype.

Bilateral uterine vessel ligation (Restricted) or sham surgery (Control) was performed on gestational day 18 (term=22 days) in rats. Post mortem of Restricted and Control mothers was performed on prenatal day 20, postnatal day 1 and 7 and weeks 5, 7 and 9, and in non-pregnant rats. Right femur dimensions, mineral content and density were measured (peripheral quantitative computed tomography; for true volumetric trabecular and cortical mineral content, density, dimensions and stress strain in).

Trabecular content and density were lower on postnatal day 1 ($p<0.05$) in Control compared to Restricted. In Control rats only, cortical bone content and density increased by prenatal day 20, following the normal profile allowing fetal skeletal mineralisation, with content falling below non-pregnant by postnatal day 1 ($p<0.05$). These deficits in trabecular and cortical bone content and density did not occur in Restricted mothers. The stress strain index of bone strength decreased in Control rats only from prenatal day 20 to postnatal day 1 ($p<0.05$). By postnatal day 7, bone parameters in both groups were not different to non-pregnant, with complete restoration of bone occurring after weaning.

Mothers exposed to uteroplacental insufficiency did not undergo the normal skeletal changes seen in Control rats. Presumably calcium supply to offspring was reduced during pregnancy and lactation, further limiting postnatal skeletal growth with potential consequences for offspring bone health. The adverse maternal bone consequences during the perinatal period were not long-lasting.

480

DIFFERENTIAL EFFECTS OF EXOGENOUS ANDROGENS AND INHIBITION OF ANDROGEN SIGNALLING IN THE DEVELOPING AND MATURE MURINE MAMMARY GLAND

L. M. Butler[1], A. A. Peters[1], W. V. Ingman[2], W. D. Tilley[1]

[1]Dame Roma Mitchell Cancer Research Laboratories, School of Medicine, University of Adelaide, Adelaide, SA, Australia
[2]Discipline of Obstetrics and Gynaecology and Research Centre for Reproductive He, University of Adelaide, SA, Australia

There is emerging evidence that androgens inhibit the proliferation of normal and malignant breast epithelial cells, and may thus counteract the proliferative actions of estrogens in the breast. However the actions of androgens on the normal mammary gland are not well understood. In this study we investigated whether the growth and development of the normal mammary gland could be altered by either stimulating or suppressing androgen signalling in vivo. Intact female mice aged 5 weeks (mid-puberty) or 12 weeks (post-puberty) were implanted with a slow-release pellet containing either placebo, 5α-dihydrotestosterone (DHT; 1mg) or the androgen receptor (AR) antagonist flutamide (60mg) for up to 9 weeks. After treatment, mammary glands were excised from each mouse for analysis. Treatment with DHT commencing mid-puberty retarded ductal extension by 40% ($P<0.01$; Mann-Whitney U test), but had no significant effect on mammary gland morphology when treatment was commenced post-puberty. In contrast, inhibition of androgen signalling with flutamide commencing mid-puberty had no effect on the mammary gland, but post-pubertally flutamide significantly increased ductal branching ($P<0.01$) and proliferation ($P=0.033$) of breast epithelial cells. Radioimmunoassays and immunohistochemistry showed that the increase in proliferation observed in mammary glands from the flutamide-treated mice was not due to an increase in E2 serum levels or estrogen receptor-alpha (ERα) expression levels. Interestingly, both the level and intensity of the AR, but not ERα, increased significantly ($P<0.01$) in the mammary gland over the period of the hormone treatments, resulting in a marked reduction in the ERα to AR ratio in mature animals. Collectively, our findings suggest that androgen signalling plays an anti-proliferative role and influences the development and structure of the normal mammary gland. Importantly, a homeostatic balance between estrogen and androgen signalling in the mature mammary gland appears to be critical to constrain the proliferative effects of estrogen. We propose that enhanced cellular proliferation associated with perturbation of androgen action in the mature mammary gland may increase the risk of malignant transformation.

481

BMPR-IB HAS A DEVELOPMENTAL ROLE IN TESTOSTERONE PRODUCTION IN MALE MICE.

I. M. Ciller, U. A. Ciller, J. R. McFarlane

Centre for Bioactive Discovery in Health and Ageing, School of Science and Tech., University of New England, Armidale, NSW, Australia

The male reproductive system is regulated by pituitary gonadotrophins in tandem with local factors including the transforming growth factor-β family members. Bone morphogenetic proteins (BMPs) and their receptors have been reported to have important roles in the modulation of testosterone synthesis via their effect on steroidogenic enzymes. In this study we investigated the effects of passive BMPR-IB neutralization on serum testosterone in immature and mature male mice to investigate the role of the type I receptor in testosterone production. Immature (21d) and adult (60d) male mice were passively immunized against BMPR-IB using subcutaneous injections of 100 µl PBS containing anti-BMPR-IB in the absence and presence of equine chorionic gonadotrophin. The preparations were administered every day for six days. On the seventh day mice were sacrificed by CO2 asphyxiation and the blood collected by cardiac puncture. Serum steroids were extracted using diethyl ether and assayed for testosterone using a radioimmunoassay. BMPR-IB immunization significantly reduced gonadotrophin-induced testosterone concentrations in immature male mice, while not significantly altering basal testosterone concentrations. In mature mice BMPR-IB neutralization significantly stimulated basal testosterone production, while having no effect on gonadotrophin-stimulated testosterone concentrations. Our research demonstrates that gonadotrophin-induced testosterone production is enhanced by ligand signalling through BMPR-IB in immature mice, while the receptor inhibits basal testosterone production in mature animals, demonstrating a developmental change in the role of BMPR-IB in the modulation of testosterone production in male mice.

482

ODC1 AND *TCEAL7* ARE POTENTIAL MEDIATORS OF ANDROGEN ACTIONS IN SKELETAL MUSCLE

N. Lee, J. D. Zajac, H. E. MacLean

Medicine (Austin Health), University of Melbourne, Heidelberg, VIC, Australia

We aim to identify target genes mediating androgen actions in muscle, using our global androgen receptor (AR) knockout (ARKO) mice, which have a 20% decrease in muscle mass[1]. We previously showed that two genes with altered expression in ARKO muscle are *Odc1* (2.6-fold decrease) and *Tceal7* (2.4-fold increase). These genes also showed a similar pattern of expression in muscle-specific ARKO mice. *Odc1* encodes ornithine decarboxylase, a regulator of proliferation[2] and a rate-limiting enzyme in polyamine biosynthesis pathway[3], and *Tceal7*, an ovarian tumor suppressor[4]. We are now investigating the expression patterns and function of these genes in muscle *in vivo* and *in vitro*.

We determined the expression of *Odc1* and *Tceal7* in muscle of pre-pubertal (4 weeks old) and adult (12 weeks old), male and female mice (n=5-12/group). *Odc1* expression is 4-fold higher in adult versus pre-pubertal males ($p=0.001$) and higher in adult males than females ($p<0.05$). *Tceal7* expression is 5-fold lower in adult versus pre-pubertal males ($p<0.01$). In a human skeletal muscle cell line (n=6-10/group), *Odc1* expression is high in proliferating myoblasts, and decreases ~90% ($p<0.05$) in differentiated myotubes. *Tceal7* expression is low in proliferating myoblasts and increases ~90% in differentiated myotubes. A similar result was observed in C2C12 mouse myoblasts and myotubes. To investigate *Odc1* function, C2C12 myoblasts were stably transfected with an *Odc1* overexpression vector or treated with an Odc1 inhibitor, α -difluoromethylornithine (DFMO). Cell proliferation is normal in C2C12 myoblasts overexpressing *Odc1*; whereas DFMO treatment inhibits myoblast proliferation by ~60% at 72hr ($p<0.001$).

Our data demonstrate that *Odc1* and *Tceal7* are developmentally regulated by androgens in adult muscle, and this occurs directly via the AR. *Odc1* is essential for muscle cell proliferation, suggesting androgens may act in part to regulate muscle cell number to increase muscle mass.

(1) HE MacLean et al. FASEB J. 22: 2676, 2008
(2) JA Nilsson et al. Cancer Cell 7: 433, 2005
(3) AE Pegg et al. Physiol. 243:C212-21, 1982
(4) J Chien et al. Oncogene 24: 5089, 2005

483

ANDROGEN REGULATION OF 5A-REDUCTASE TYPE 2 IN THE MOUSE PROSTATE

K. M. McNamara, D. T. Harwood, U. Simanainen, D. J. Handelsman

Andrology, ANZAC Research Institute, Concord, NSW, Australia

The prostate is highly androgen dependent and the principal tissue expressing 5α-Reductase type 2(5aR2) in males. While rat ventral prostate 5αR2 is positively regulated by androgens[1], its regulation has been little examined in the mouse. We used variable androgen receptor(AR) expression in different prostate lobes to determine if 5αR2 expression and tissue DHT levels correlated with expression of AR in the lobes. Therefore, 5αR2 mRNA (via real-time RT-PCR) and DHT (via LC-MSMS, the functional product of 5αR2 activity) were compared to AR expression in the anterior prostate(AP), ventral prostate(VP) and dorsolateral prostate(DLP) of the normal mouse. Prostate 5αR2 mRNA and DHT levels were lowest in the AP followed by the DLP and VP and coinciding with the rank order of epithelial AR immunopositivity(lowest in the AP followed by the DLP and VP) suggesting the epithelial AR may positively regulate the gene expression and activity of 5αR2. To further test this theory we used the Prostate Epithelial Androgen Receptor Knockout(PEARKO) mouse model to examine the effect of epithelial AR deprivation on 5αR2. Unexpectedly, the selective inactivation of epithelial AR lead to significantly

increased 5αR2 mRNA and DHT levels when compared to control AP, while castration returned the 5αR2 mRNA in the AP of the PEARKO to control levels. Immunohistochemistry revealed that the strong 5αR2 expression in the PEARKO AP was localised to areas of abnormal epithelial cell clusters seen in the PEARKO AP. In summary epithelial AR appears to have a role in regulating 5αR2 expression as seen by the increase epithelial AR immunopositivity and 5αR2 expression in PEARKO. However stromal AR and/or other factors may also have a significant role as castration reduced the 5αR2 expression in PEARKO. Finally the enzyme localisation suggests the increased 5αR2 in the AP of PEARKO is possibly related to abnormalities in epithelial differentiation.

(1) J.M. Torres, E. Ruiz, E. Ortega, Development of a quantitative RT-PCR method to study 5alpha-reductase mRNA isozymes in rat prostate in different androgen status, Prostate 56(1) (2003) 74-79.

484

ANDROGEN RECEPTOR-MEDIATED ACTIONS PLAY A ROLE IN REGULATING LATE FOLLICULAR DYNAMICS AND EMBRYO DEVELOPMENT PAST THE 2-CELL STAGE

K. A. Walters, S. R. Joseph, X. Cheng, C. M. Allan, D. J. Handelsman

Andrology, ANZAC Research Institute, Sydney, NSW, Australia

Recently the androgen receptor (AR) has been shown definitively to play a role in female reproduction (*1*) . We generated homozygous $AR^{-/-}$ female mice using Cre/LoxP recombination for an in-frame excision of exon 3, encoding the second zinc finger essential for DNA-binding. $AR^{-/-}$ females are sub-fertile with reduced ovulation rates, however, this was overcome by gonadotrophin hyperstimulation, suggesting a defect in hypothalamic-pituitary regulation of ovulation (*2*) . Ovary transplantation studies identified that the sub-fertility is due to both intrinsic ovarian defects and a disruption in extra-ovarian hypothalamic-pituitary regulatory mechanisms (*3*) . In this study we examined further the intra-ovarian defects. $AR^{-/-}$ and control ($AR^{+/+}$) ovaries collected at proestrus showed no difference in antral follicle numbers, however $AR^{-/-}$ ovaries exhibited fewer preovulatory follicles compared to $AR^{+/+}$ ovaries ($AR^{-/-}$: 1.8 ± 0.5 ; $AR^{+/+}$: 5.2 ± 0.9, $P<0.01$) . $AR^{-/-}$ small antral follicles had a reduced oocyte:follicle ratio ($AR^{-/-}$: 0.3 ± 0.004 ; $AR^{+/+}$: 0.4 ± 0.007, $P<0.01$) , indicating a disruption in the connection between the oocyte and somatic cells and an altered pattern of growth. There was a 14% increase in the percentage of unhealthy large antral follicles (>10% pyknotic granulosa cells) present in the $AR^{-/-}$ ovaries ($P<0.05$) . Additionally, $AR^{-/-}$ oocytes collected on the identification of a copulatory plug, after overnight housing of females with a fertile male, exhibited disorganised and sparse cumulus cells compared to $AR^{+/+}$ oocytes. Fewer embryos were collected from $AR^{-/-}$ females ($AR^{-/-}$: 6.8 ± 0.9 ; $AR^{+/+}$:11.1 ± 1.6, $P<0.05$), and the percentage of $AR^{-/-}$ fertilized oocytes which progressed to morula (73% decrease) and blastocyst (72% decrease) developmental stages over a 5 day culture was significantly reduced compared to $AR^{+/+}$ embryos ($P<0.01$). In conclusion, reduced ovulations observed in $AR^{-/-}$ females is due, at least in part, to reduced late follicle health, leading to the development of fewer preovulatory follicles and a disruption in AR signaling, effecting embryo quality.

(1) K. A. Walters, C. M. Allan, D. J. Handelsman, Biol. Reprod. 78, 380 (2008).
(2) K. A. Walters et al., Endocrinology 148, 3674 (2007).
(3) K. A. Walters et al., Endocrinology 150, 3274 (2009).

485

TGF-BETAS AND ACTIVIN REGULATION OF STEROIDOGENESIS IN TM3 LEYDIG CELLS IS SMAD2-DEPENDENT

Y. Wang, M. Bilandzic, G. T. Ooi, J. K. Findlay, K. L. Stenvers

Female Reproductive Biology, Prince Henry's Institute of Medical Research, Victoria, VIC, Australia

TGF-beta superfamily members are suspected regulators of Leydig cell development and function. To determine specific roles for individual TGF-beta superfamily members in Leydig cells, we utilise an *in vitro* model of immature murine Leydig cells, the TM3 cell line, which expresses the receptors and downstream effectors of several TGF-beta superfamily members. We have previously shown that both TGF-beta and activin inhibit steroidogenesis in these cells and that knockdown of *Inha* reduced the expression of several genes involved in steroidogenesis by 40-80% ($p<0.05$), presumably due to increased endogenous activin activity (Wang et al., Abstract#245, ESA 2009). However, the signalling pathways by which these growth factors regulate steroidogenesis are poorly understood. The objective of this study was to test the hypothesis that TGF-beta and activin regulate steroidogenesis in TM3 cells via activation of downstream SMAD molecules. Western blot analyses using activation-specific antibodies against the SMAD molecules revealed that the TM3 cells exhibited up to a 35-fold activation of SMAD2, but not SMAD3, in response to either activin A (2 nM), TGF-beta1 (40 pM), or TGF-beta2 (40 pM). These results were confirmed by luciferase reporter assays using SMAD2- and SMAD3-sensitive reporter constructs (pAR3-lux and pGL3-(CAGA)12-Luc, respectively), wherein TGF-beta or activin A only stimulated SMAD2 reporter activity. Surprisingly, knockdown of *Inha* expression enhanced both activin A and TGF-beta induced SMAD2 activation by 2-4 fold, suggesting that autocrine inhibins may antagonise both classes of growth factor. A chemical inhibitor of the TGF-beta/activin type I receptors, SB431542, completely blocked TGF-beta- or activin-mediated SMAD2 activation and downregulation of *Cyp17a1* luciferase reporter activity. Collectively, our data indicate that in TM3 cells, both activin and TGF-betas negatively regulate steroidogenesis via activation of their type I receptors and SMAD2. Supported by the NHMRC Australia (#494802; 441101; 388904) and Victorian Government Infrastructure funds.

(1) Yao Wang, Jock K. Findlay, and Kaye Stenvers. Autocrine inhibins regulate immature mouse Leydig cell behaviour. Abstract #245, ESA, 2009

Shaffinaz Abd Rahman
The University of Queensland, QLD
s.abdrahman@uq.edu.au

Sarah Abdo
St Vincents Hospital/Garvan Institute, NSW
sarahabdo@hotmail.com

Naomi Achong
RBWH, QLD
missnomy@hotmail.com

Reidun Aesoey
Monash University, VIC
Reidun.Aesoey@monash.edu

Isaac Ahimah
, Lome
Isaac@arg-eportal.org

Ashraf Ahmed
Princess Alexandra Hospital, QLD
ashraf.uq@gmail.com

Robert Aitken
Newcastle University, NSW
John.Aitken@newcastle.edu.au

Lisa Akison
Robinson Institute, Research Centre for Reproductive Health, University of Adelaide, SA
lisa.akison@adelaide.edu.au

Jasna Aleksova
St Vincents Hospital, VIC
jasnaaleksova@hotmail.com

Kate Alger
Novo Nordisk, NSW
kalg@novonordisk.com

Sinan Ali
Macquarie University, NSW
sinan.ali@mq.edu.au

Carolyn Allan
Prince Henry's Institute, VIC
Carolyn.Allan@princehenrys.org

Charles Allan
ANZAC Research Institute/University of Sydney, NSW
charles@med.usyd.edu.au

Gloria Anampiu
, Lome
gloria@arg-eportal.org

Amanda Anderson
The University of Newcastle, NSW
Amanda.Anderson@newcastle.edu.au

Prabha Andraweera
University of Adelaide, SA
prabha.andraweera@adelaide.edu.au

Daniel Anghel
American College of Cardiology, NY
danghel1982@aol.com

Algenes Aranha
Logan Beaudesert Diebetes Servive, QLD
Algenes_Aranha@health.qld.gov.au

Nirusha Arnold
Private Practice, NSW
nirusha_arnold@ozemail.com.au

Helen Atkinson
University of Western Australia, WA
helen.atkinson@uwa.edu.au

Shaikh Irfan Aziz
The Queen Elizabeth Hospital, SA
irfandmc@yahoo.com

Leon Bach
The Alfred, VIC
Leon.Bach@monash.edu

Warwick Bagg
University of Auckland,
w.bagg@auckland.ac.nz

Hassan Bakos
Repromed, SA
hassan.bakos@adelaide.edu.au

Jean-Claude Balducci
SciGen (Australia) Pty Ltd, NSW
jcbalducci@scigen.com.au

Helen Barrett
Royal Brisbane and Women, QLD
hbarrett@gmp.usyd.edu.au

Miriam Bartlett
Western Health, VIC
mbartlett@optusnet.com.au

Paul Bartley
Stoneham Chambers, QLD
paulcbartley@bigpond.com.au

Kiri Beilby
The University of Sydney, NSW
k.beilby@usyd.edu.au

Katherine Benson
Concord Hospital, NSW
katherinebenson@iinet.net.au

Daniel Bernet
Servier, VIC
daniel.bernet@au.netgrs.com

Ilana Berntstein
The University of Newcastle, NSW
Ilana.Bernstein@newcastle.edu.au

Michael Bertoldo
University of Sydney, NSW
m.bertoldo@usyd.edu.au

Maree Bilandzic
Prince Henry's Insitute , VIC
maree.bilandzic@princehenrys.org

Natalie Binder
The University of Melbourne, Vic
nbinder@pgrad.unimelb.edu.au

Vita Birzniece
Garvan Institute of Medical Research, NSW
v.birzniece@garvan.org.au

Helen Blanchfield
Novo Nordisk Pharmaceuticals, QLD
hebd@novonordisk.com

Frank Bloomfield
University of Auckland, Ack
f.bloomfield@auckland.ac.nz

Sascha Boldt
Novartis, NSW
sascha.boldt@novartis.com

Maria Rosa Borges
#433 Rue Aguolu, Lome
maria@arg-eportal.org

Michael Bramble
MSD, NSW
michael.bramble@merck.com

Wilton Braund
Tennyson Centre, SA
wbraund@doctorsrooms.com

Dana Briggs
Monash University, VIC
dana.briggs@monash.edu

Kara Britt
Monash University, VIC
kara.britt@monash.edu

Morton Burt
Flinders Medical Centre, SA
morton.burt@health.sa.gov.au

Lisa Butler
University of Adelaide DRMCRL, SA
marie.a.pickering@gmail.com

Noreen Cahill
Novo Nordisk, NSW
noca@novonordisk.com

Natalie Calatayud
Melboirne University, VIC
nec@unimelb.edu.au

Pamela Callum
California Cryobank, CA
p_callum@hotmail.com

Donald Cameron
Brisbane Clinic, QLD
don_cam@bigpond.net.au

Kirsten Campbell
Flinders Medical Centre, SA
drkirstcampbell@gmail.com

Carmela Caputo
St Vincent's Hospital, Vic
carmela.caputo@svhm.org.au

Ian Caterson
, NSW
ian.caterson@sydney.edu.au

Tom Cawood
Christchurch Hospital, CHCH
tom.cawood@cdhb.govt.nz

Bree Cawsey
Monash University, VIC
bree.cawsey@monash.edu.au

Jackie Center
Garvan Institute of Medical Research and St Vincent's Hospital, NSW
j.center@garvan.org.au

Joshua Chaffin
#433 Rue Aguolu, Lome
joshua@arg-eportal.org

Sze Yee Chai
MBRC/HMRI, NSW
cszeyee@gmail.com

Larry Chamley
University of Auckland, N/A
l.chamley@auckland.ac.nz

Heng Chun Chan
Chan Medical & Endocrine Clinic,
drhcchan@hotmail.com

Katie Chan
Sapphire Bioscience Pty. Ltd., NSW
katiec@sapphirebioscience.com

Santosh Chaubey
The Townsville Hospital, Townsville, QLD
drchaubeysk@rediffmail.com

Qi Chen
The University of Auckland, AKL
q.chen@auckland.ac.nz

YuXia Chen
The University of Newcastle, NSW
yuxia.chen@newcastle.edu.au

Daniel Chen
St Vincent's Hospital, NSW
silverchance@hotmail.com

Yi Chen
Monash Institute of Medical Research, VIC
chen_yi11@hotmail.com

Xiaobing Cheng
ANZAC Research Institute, NSW
xcheng@anzac.edu.au

Ada Cheung
Royal Melbourne Hospital, VIC
adacheung@hotmail.com

Yu-Yan, Chloe Cheung
The University of Hong Kong, HK
ycc106@hkusua.hku.hk

Keng Yih Chew
The University of Melbourne, Vic
kychew@unimelb.edu.au

Karen Chiam
University of Adelaide/ Dame Roma Mitchell Cancer Research Laboratories, SA
karen.chiam@adelaide.edu.au

Viral Chikani
Gold Coast Hospital, QLD
viralchikani@hotmail.com

Peck Yin Chin
University of Adelaide, SA
p.y.chin@adelaide.edu.au

David Chipps
Western Sydney Diabetes and Endocrine Centre, NSW
dchipps@med.usyd.edu.au

Sridhar Chitturi
Royal Darwin Hospital, NT
srichitturi@hotmail.com

Suyinn Chong
Queensland Institute of Medical Research, QLD
Suyinn.Chong@qimr.edu.au

Catherine Choong
Princess Margaret Hospital, WA
catherine.choong@health.wa.gov.au

Nik Chosich
Alfred Hospital, Vic
nikchos@tpg.com.au

Simon Chu
Prince Henry's Institute, VIC
simon.chu@princehenrys.org

Wing Hong Vincent Chu
University of Adelaide, SA
vincent.chu@adelaide.edu.au

Jodi Clancy-Stott
Novo Nordisk, NSW
jocl@novonordisk.com

Iain Clarke
Monash University, Vic
iain.clarke@monash.edu

Christine Clarke
Westmead Institute for Cancer Research, NSW
christine.clarke@sydney.edu.au

Peter Clifton
CSIRO Human Nutrition, SA
Peter.Clifton@csiro.au

Roderick Clifton-Bligh
Royal North Shore Hospital, NSW
jclifton@med.usyd.edu.au

Timothy Cole
Monash University, Vic
tim.cole@monash.edu

Peter Colman
Royal Melbourne Hospital, VIC
peter.colman@mh.org.au

Ailie Connell
Alfred Health, VIC
ailie_connell@hotmail.com

Halina Connelly
Servier, VIC
halina.connelly@au.netgrs.com

Catherine Conway
Canterbury District Health Board,
hlavacconway@xtra.co.nz

Sheila Cook
Toowoomba Hospital, Qld
as_cook@telstra.com.au

Davina Cossigny
Prince Henry's Instituteof Medical Research, Vic
davina.cossigny@princehenrys.org

Rachael Crew
The University of Western Australia, WA
20269125@student.uwa.edu.au

Jim Cummins
Murdoch University, WA
j.cummins@murdoch.edu.au

Sarah Dalati
University of Sydney, NSW
sdal7726@uni.sydney.edu.au

Abhay Daniel
Royal Brisbane Hospital, QLD
abhaydaniel2003@yahoo.com

Deborah Davis
Medtronic, NSW
deborah.davis@medtronic.com

Margot Day
Sydney University, NSW
margotd@physiol.usyd.edu.au

Miles De Blasio
The University of Adelaide, SA
miles.deblasio@adelaide.edu.au

Ralph De Fronzo
University of Texas, TX

ALBARADO@uthscsa.edu

Kathleen Deboer
Monash University (Clayton campus), VIC
kathleen.deboer@med.monash.edu.au

John Delahunt
University of Otago, Wellington,
john.delahunt@otago.ac.nz

Barbara Depczynski
Liverpool Hospital, NSW
barbara.depczynski@sswahs.nsw.gov.au

Reena Desai
ANZAC Reserach Institute, NSW
reenadesai@med.usyd.edu.au

Marianne Diaz
Institute For Molecular Bioscience, Qld
m.diaz@uq.edu.au

Evdokia Dimitriadis
Prince Henry's Institute of Medical Research, VIC
Evdokia.Dimitriadis@princehenrys.org

Kim Donaghue
The Children's Hospital at Westmead, NSW
kimd@chw.edu.au

Hollian Douglas
University of Otago, n/a
hollian.douglas@anatomy.otago.ac.nz

Samson Dowland
The University of Sydney, NSW
sdowland@anatomy.usyd.edu.au

Ann Drummond
Prince Henry's Institute of Medical Research, Victo
ann.drummond@princehenrys.org

Matt Dun
The University of Newcastle, NSW
Matt.Dun@uon.edu.au

Emma Duncan
Royal Brisbane and Women's Hospital, QLD
e.duncan@uq.edu.au

Kylie Dunning
University of Adelaide, SA
kylie.dunning@adelaide.edu.au

Kemperly Dynon
Prince Henry's Institute of Medical Research, VIC
kemperly.dynon@princehenrys.org

Rebecca Dyson
Mothers & Babies Research Centre, NSW
rebecca.dyson@uon.edu.au

Creswell Eastman
Sydney Thyroid Clinic, NSW
eastcje@ozemail.com.au

M.Jane Ellis
Christchurch Hospital, NZ
jane.ellis@cdhb.govt.nz

Jacob Engwerda
#433 Rue Aguolu, Lome
Jacob@arg-eportal.org

Pablo Enriori
Monash University, VIC
pablo.enriori@monash.edu

Kemal M. Erbil
GATA, ANKAR
mcihan@gmail.com

Natalie Eriksson
University of Queensland, QLD
n.eriksson@uq.edu.au

Jemma Evans
Prince Henry\'s Institute of Medical Research, VIC
jemma.evans@princehenrys.org

Jim Fawcett
ASN Events, VIC
jf@asnevents.net.au

Gerry Fegan
Fremantle Hopsital, WA
gerry.fegan@health.wa.gov.au

Jane Fenelon
The University of Melbourne, Vic
j.fenelon@pgrad.unimelb.edu.au

Jock Findlay
Prince Henry's Institute of Medical Research, Vic
jock.findlay@princehenrys.org

Daniel Fineberg
Alfred Health, VIC
daniel.fineberg@med.monash.edu.au

Rebecca Fitzsimmons
Institute for Molecular Bioscience, Qld
r.fitzsimmons@uq.edu.au

Tony Flint
University of Nottingham, Leics
anthony.flint@nottingham.ac.uk

Paul Fogarty
Pregnancy and Development Group Robinson Institute Lyell McEwin Hospital University of Adelaide, SA
paul.fogarty@adelaide.edu.au

Vincent Fong
Private, NSW
vsjhfracp@yahoo.com.au

Spiros Fourlanos
Royal Melbourne & Northern Hospitals, VIC
spiros.fourlanos@mh.org.au

Jayne Franklyn
University of Birmingham, BM
j.a.franklyn@bham.ac.uk

Peter Fuller
Prince henrys Institute of Medical Research, Vic
peter.fuller@princehenrys.org

Denise Furness
The Robinson Institute, University of Adelaide, SA
denise.furness@adelaide.edu.au

Bart Gadella
Utrecht University, TC
B.M.Gadella@uu.nl

Lucy Gagliardi
Royal Adelaide Hospital, SA
lucia.gagliardi@health.sa.gov.au

Melissa Gamat
The University of Melbourne, Vic
m.gamat@pgrad.unimelb.edu.au

Lakshi Ganeshan
University of Sydney, NSW
lakshi.ganeshan@sydney.edu.au

Yan Ru Gao
Anzac Research Institute, NSW
egao@anzac.edu.au

David Gardner
University of Melbourne, VIC
david.gardner@unimelb.edu.au

Caroline Gargett
Monash Institute of Medical Research, VIC
caroline.gargett@monash.edu

Kathy Gatford
University of Adelaide, SA
kathy.gatford@adelaide.edu.au

Rachel Gentles
University of Sydney, NSW
rachel.gentles@sydney.edu.au

Paul Georgiou
Royal Adelaide Hospital, SA
paul.georgiou@health.sa.gov.au

Emily Gianatti
Austin Health, VIC
emily.gianatti@gmail.com

Iwona Gileff
Flinders Medical Centre, SA
iwonagileff@hotmail.com

Chris Gilfillan
Eastern Health, Victo
chris.gilfillan@easternhealth.org.au

Jane Girling
Monash Institute of Medical Research, VIC
jane.girling@med.monash.edu.au

Sarah Glastras
Royal North Shore Hospital, NSW
sarahglastras@gmail.com

Michelle Gordon
Peninsula Health, Vic
amgordon@bigpond.net.au

Patricia Grant
University of Adelaide, SA
patricia.grant@adelaide.edu.au

Mathis Grossmann
University of Melbourne Austin Health, VIC
mathisg@unimelb.edu.au

Christopher Grupen
The University of Sydney, NSW
christopher.grupen@sydney.edu.au

Frank Grutzner
The University of Adelaide, SA
frank.grutzner@adelaide.edu.au

Neil Gude
Royal Women's Hospital, VIC
neil.gude@thewomens.org.au

Kara Gunter
The University of Newcastle, NSW
Kara.Gunter@uon.edu.au

Caroline Hadley
CSIRO Publishing,
caroline.hadley@csiro.au

Judy Hadwen
PA Hospital, QLD
judehadwen@hotmail.com

David Handelsman
ANZAC Research Institute, NSW
djh@anzac.edu.au

Jessica Harris
Institute of Health and Biomedical Innovation, QLD
j1.harris@connect.qut.edu.au

Natalie Harrison
Geelong Hosptial, Vic
drnatalieharrison@gmail.com

Himawan Harryanto
The University of Adelaide, SA
ker.cheah@adelaide.edu.au

Terry Hassold
Washington State University, WA
terryhassold@wsu.edu

Nick Hatzirodos
University of Adelaide, SA
nicholas.hatzirodos@adelaide.edu.au

Jenna Haverfield
Prince Henry's Institute of Medical Research, VIC
jenna.haverfield@princehenrys.org

Penny Hawken
University of Western Australia, WA
phawken@animals.uwa.edu.au

Lisa Hayes
Royal Brisbane and Women's Hospital, QLD
lisahayes99@gmail.com

John Hearn
The University of Sydney, NSW
sheelagh.douglas@sydney.edu.au

Mark Hedger
Monash University, VIC
mark.hedger@med.monash.edu.au

Sophea Heng
Prince Henry's Institute of Medical Research, VIC
sophea.heng@princehenrys.org

David Henley
Sir Charles Gairdner Hospital, WA
dhenley@cygnus.uwa.edu.au

Belinda Henry
Monash University, VIC
belinda.henry@monash.edu

Theresa Hickey
University of Adelaide, SA
marie.a.pickering@gmail.com.au

Amanda Highet
University of Adelaide, SA
amanda.highet@adelaide.edu.au

Jonathan Hirst
University of Newcastle, NSW
jon.hirst@newcastle.edu.au

Tien-Ming Hng
Blacktown Hospital, NSW
tienming@bigpond.net.au

Jean Ho
Royal Prince Alfred Hospital, NSW
jonflee8000@yahoo.com.au

Ken Ho
Garvan Institute of Medical Research, NSW
k.ho@garvan.org.au

Kenneth Ho
Ryde Hospital, NSW
kennethwaikheongho@yahoo.com.au

Huiting Ho
Prince Henry's Institute, VIC
huiting.ho@princehenrys.org

Jeremy Hoang
Gosford Hospital, NSW
jqhoang@gmail.com

Nicolette Hodyl
Robinson Institute, University of Adelaide, SA
nicolette.hodyl@adelaide.edu.au

Jane Holmes-Walker
Westmead Hospital, NSW
jane.holmeswalker@sydney.edu.au

Lorissa Hopkins
The University of Newcastle, NSW
Lorissa.Hopkins@uon.edu.au

Louise Howson
Novartis Pharmaceuticals, NSW
louise.howson@novartis.com

Kiernan Hughes
Royal Prince Alfred, NSW
kiernanhughes@gmail.com

Katja Hummitzsch
The University of Adelaide, SA
katja.hummitzsch@adelaide.edu.au

Patricia Hunt
Washington State University, WA
pathunt@wsu.edu

Shirin Hussain
Monash University, VIC
shus3@student.monash.edu.au

Jennifer Ly Huynh
Monash University Department of Biochemistry & Molecular Biology, VIC
Jennifer.Huynh@monash.edu

Jenny Huynh
Austin Health, Vic
Huynh_Jenny@hotmail.com

Zoe Hyde
University of Western Australia, WA
zoe@sexologyresearch.org

Warrick Inder
St Vincent's Hospital, VIC
winder@medstv.unimelb.edu.au

Wendy Ingman
University of Adelaide, SA
wendy.ingman@adelaide.edu.au

Helen Irving-Rodgers
The University of Adelaide, SA
helen.irvingrodgers@adelaide.edu.au

Stacey Jamieson
Prince Henry's Institute, VIC
Stacey.Jamieson@princehenrys.org

Duangporn Jamsai
Monash University, VIC
Duangporn.Jamsai@med.monash.edu.au

Christina Jang
St Vincent's Hospital, VIC
christina.jang@svhm.org.au

Phoebe Jennings
University of Newcastle, NSW

phoebe.jennings@newcastle.edu.au

George Jerums
Austin Health, Vic
g.jerums@unimelb.edu.au

Xingliang Jin
University of Sydney, NSW
xingliang.jin@sydney.edu.au

Anju Joham
Southern Health, VIC
ajoham@hotmail.com

Mohammad Zahied Johan
Research Centre for Reproductive Health, SA
mohammad.johan@adelaide.edu.au

Kalle Jonasson
, Lome
kalle@arg-eportal.org

Graeme Jones
University of Tasmania, TAS
Graeme.Jones@utas.edu.au

Keith Jones
University of Newcastle, NSW
keith.jones@newcastle.edu.au

Shai Joseph
Anzac Research Institute, NSW
sjoseph@anzac.edu.au

Shantha Joseph
Flinders Medical Center, SA
shantharose76@yahoo.com

Devina Joshi
Westmead Hospital, NSW
devinadjoshi@gmail.com

Kishani Kannangara
Prince Henry's Institute / Monash Medical Centre, VIC
kkannang@med.usyd.edu.au

Nicholas Kasmeridis
Flinders Medical Centre, SA
nkasmeri@gmail.com

Leah Katsoulotos
Private Practice, NSW
drhlk@tpg.com.au

Victor Kaydor
, Lome
support@arg-eportal.org

Meredith Kelleher
Mothers & Babies Research Centre, University of Newcastle, NSW
meredith.kelleher@uon.edu.au

Dean Kelly
Mater Pathology, Qld
dean.kelly@mater.org.au

Katherine Kibbey
Geelong Hospital, VIC
katherinekibbey@hotmail.com

Kevin Knower
Prince Henry's Institute, VIC
kevin.knower@princehenrys.org

John Kok
Westmead Hospital, NSW
johnkok@doctor.com

Wee-Ching Kong
University of Adelaide, SA
weeching.kong@adelaide.edu.au

Adam Koppers
Monash University, VIC
adam.koppers@monash.edu.au

Mark Kotowicz
The University of Melbourne, Vic
markk@BarwonHealth.org.au

Kim Ku
Sunnybank/Greenslope, QLD
yeekimku@gmail.com

Monica Kwan
Private Practice, SA
hk_lam@yahoo.com

Narsing Rao Laddipeerla
TQEH, SA
drnarsing@gmail.com

Sarah Lamb
ANZAC Research Institute, NSW
slamb@anzac.edu.au

Michelle Lane
Repromed/Adelaide Uni, SA
mlane@repromed.com.au

Namson Lau
BIONE, NSW
nsclau@yahoo.com

Sue Lynn Lau
Garvan Institute, NSW
sllau@hotmail.com

Florence Law
Private Practice, NSW
flo_law@yahoo.com

Syndia Lazarus
Mater Hospital, QLD
syndia.lazarus@gmail.com

Kyren Lazarus
Prince Henrys Institute, VIC
lazaruskyren@hotmail.com

Diana Learoyd
Royal North Shore Hospital, NSW
diana.learoyd@sydney.edu.au

I-Lynn Lee
Western Hospital, VIC
ilynnlee@hotmail.com

Heather Lee
Garvan Institute of Medical Research, NSW
heather.lee@garvan.org.au

Paul Lee
Garvan Institute of Medical Research, NSW
p.lee@garvan.org.au

Nicole KL Lee
University of Melbourne (Austin Health), VIC
k.lee10@pgrad.unimelb.edu.au

Peter Leedman
Western Australian Institute for Medical Research, WA
peterl@waimr.uwa.edu.au

Mridula Lewis
Southern Endocrine, NSW
mridula.lewis@doctor.com

Joshua Lewis
Sir Charles Gairdner Hospital, WA
josh.lewis@meddent.uwa.edu.au

Jessica Lewis
The University of Western Australia, WA
jessica.lewis@uwa.edu.au

Ying Li
Prince Henry's Institute of Medical Research, VIC
ying.li@princehenrys.org

Yan Li
University of Sydney, NSW
yan.l@sydney.edu.au

Qun Li
Monash University, VIC
qun.li@monash.edu

Stafford Lightman
University of Bristol, BS
Stafford.Lightman@bristol.ac.uk

Anna Lih
Department of Endocrinology, NSW
annaklih@gmail.com

Matthew Lilse
Novo Nordisk, NSW
mwli@novonordisk.com

Richard Lim
University of Technology Sydney, NSW
Richard.Lim@uts.edu.au

Ee Mun Lim
PathWest & Sir Charles Gairdner Hospital, WA
eemun.lim@health.wa.gov.au

Shu Ly Lim
University of Adelaide, SA

shu.lim@adelaide.edu.au

Jia Yi (Joy) Lin
Victorica University of Wellington, NZ
joy.lin@vuw.ac.nz

Laura Lindsay
The University of Sydney, NSW
laural@anatomy.usyd.edu.au

Stuart Linton
Deakin University, VIC
stuart.linton@deakin.edu.au

Peter Liu
Woolcock Institute of Medical Research, University of Sydney, NSW
pliu@mail.usyd.edu.au

Hong Liu
The Liggins Institute, NZ
trkstr@gmail.com

Chi Yi Lo
Monash University, VIC
jennifer.lo@med.monash.edu.au

Dishan Lowe
St John of God Hospital, Victo
wadilowe@yahoo.co.uk

Eugenie Lumbers
University of nEwcastle, NSW
eugenie.lumbers@newcastle.edu.au

Judy Luu
Hunter New England Health, NSW
judoluu@gmail.com

Isabelle Lys
Massey University, NZ
i.lys@massey.ac.nz

James Madden
ASN Events, VIC
jm@asnevents.net.au

Gemma Madsen
MBRC/HMRI, NSW
Gemma.Madsen@newcastle.edu.au

Kaushik Maiti
MBRC, John Hunter Hospital, NSW
kaushik.maiti@newcastle.edu.au

Maria Makrides
Women's and Children's Health Research Institute, SA
Maria.Makrides@health.sa.gov.au

Irek Malecki
University of Western Australia, WA
ireneusz.malecki@uwa.edu.au

Peter Mark
The University of Western Australia, WA
peter.mark@uwa.edu.au

Tania Markovic
Royal Prince Alfred Hospital, NSW
tania_markovic@ozemail.com.au

Francine Marques
University of Sydney, NSW
fmarques@medsci.usyd.edu.au

Graeme Martin
University of WA, WA
Graeme.Martin@uwa.edu.au

Rebecca S Mason
University of Sydney, NSW
rebeccam@physiol.usyd.edu.au

Kati Matthiesson
Prince Henrys Institute, VIC
kati.matthiesson@princehenrys.org

Tracey McDonnell
Merck Serono Australia, NSW
tracey.mcdonnell@merckserono.net

Hayden McEwen
University of Otago, Otago
hayden.mcewen@anatomy.otago.ac.nz

Jim McFarlane
University of New England, NSW
jmcfarla@une.edu.au

Skye McIver
The University of Newcastle, NSW
Skye.McIver@uon.edu.au

Eileen McLaughlin
University of Newcastle, NSW
eileen.mclaughlin@newcastle.edu.au

Nigel McMillan
University of Queensland, QLD
n.mcmillan@uq.edu.au

Michael McNamara
Western Hospital Footscray, VIC
micmac79@mac.com

Keely McNamara
ANZAC Research Institute, NSW
keely.mcnamara@UNSWalumni.com

Francoise McPherson
Charles Sturt University, NSW
fmcpherson@csu.edu.au

Sarah Meachem
Prince Henrys Institute, VIC
sarah.meachem@princehenrys.org

Ellen Menkhorst
Prince Henry, VIC
ellen.menkhorst@princehenrys.org

Caroline Meyer
St Vincents Hospital, VIC
cmeyer0@bigpond.net.au

Stuart Meyers
School of Veterinary Medicine, University of California, CA
smeyers@ucdavis.edu

Megan Mitchell
University of Adelaide, SA
megan.mitchell@adelaide.edu.au

Carolyn Mitchell
Mothers and Babies Research Centre, NSW
carolyn.mitchell@newcastle.edu.au

Ezani Mohamed Jamil
University of Adelaide, SA
ezani.mohamedjamil@adelaide.edu.au

Saidatul Mohammad
The University of Adelaide, SA
saidatul.mohammad@adelaide.edu.au

Bidhu Mohapatra
Royal Adelaide Hospital, SA
bidhu_m@yahoo.com

Naswrin Moin
St George Hospital, NSW
naswrinmoin@hotmail.com

Grant Montgomery
Queensland Institute of Medical Research, QLD
grant.montgomery@qimr.edu.au

F Hamish Morgan
Prince Henry's Institute, VIC
hamish.morgan@princehenrys.org

Guillaume Morin
Monash University, VIC
stfrancoisdassise@hotmail.com

Margaret Morris
University of New South Wales, NSW
m.morris@unsw.edu.au

Sharon Mortimer
Oozoa Biomedical, BC
sharon@oozoa.com

David Mortimer
Oozoa Biomedical Inc, BC
david@oozoa.com

Farid Moslemipur
Gonbad Higher Education Center, Goles
farid.moslemipur@gmail.com

Nadarajah Mugunthan
Gold Coast Hospital, QLD
mugunthan66@hotmail.com

Beverly Muhlhausler
The University of Adelaide, SA
Beverly.Muhlhausler@adelaide.edu.au

Kirsten Murray
John Hunter Hospital, NSW
kirsten.murray@ozemail.com.au

George Muscat

Institute for Molecular Bioscience, University of Queensland, QLD
g.muscat@uq.edu.au

Peter Musya
#433 Rue Aguolu, Lome
Peter@arg-eportal.org

Vernon Naidu
Private Practice, NSW
vernonnaidu@hotmail.com

Nirjhar Nandi
Royal darwin Hospital, NT
nirjhar.nandi@nt.gov.au

Rashmi Narayanan
Concord Repatriation General Hospital, NSW
t_scrumptious@hotmail.com

Lyn-Sharon Nash
Medtronic, NSW
lyn.nash@medtronic.com

Kong Wah Ng
St Vincent\'s Hospital, Vic
kongwn@unimelb.edu.au

Jenny Ng
Southern Health, VIC
jyng@bigpond.net.au

Beverly Cheok Kuan Ng
Garvan Institute, NSW
beefooli@gmail.com

Mark Ng Tang Fui
Austin Health, 3084
markntf@hotmail.com

Rick Nicholson
John Hunter Hospital, NSW
Rick.Nicholson@hnehealth.nsw.gov.au

Guiying Nie
Prince Henry's Institute of Medical Research, VIC
guiying.nie@princehenrys.org

Brett Nixon
The University of Newcastle, NSW
brett.nixon@newcastle.edu.au

Belinda Nixon
The University of Newcastle, NSW
Belinda.Nixon@uon.edu.au

Christopher Nordin
Royal Adelaide Hospital, SA
Christopher.Nordin@health.sa.gov.au

Fleur Oback
AgResearch, n/a
fleur.oback@agresearch.co.nz

Moira O'Bryan
Monash University, Vic
moira.obryan@monash.edu

Rebecca ODonnell
Novartis Pharmaceuticals, NSW
rebecca.odonnell@novartis.com

Brian Oldfield
Monash University, VIC
brian.oldfield@monash.edu

Shaun O'Mara
Novo Nordisk, NSW
shom@novonordisk.com

Chris O'Neill
University of Sydney, NSW
chris.oneill@sydney.edu.au

Greg Ong
Sir Charles Gairdner Hospital, WA
he34213@health.wa.gov.au

Nicole Opie
Liverpool Hospital, NSW
nicole.opie@gmail.com

Tony OSullivan
University of New South Wales, NSW
A.OSullivan@unsw.edu.au

Karel Pacak
NICHD, MD
karel@mail.nih.gov

Mark Pace
Alfred Health, VIC
markdavidpace@yahoo.com.au

Leanne Pacella
Adelaide University, SA
leanne.pacella@adelaide.edu.au

Jodie Painter
Queensland Institute of Medical Research, QLD
jodie.painter@qimr.edu.au

Hannah Palliser
University of Newcastle, NSW
hannah.palliser@newcastle.edu.au

Nicole Palmer
University of Adelaide, SA
nicole.palmer@adelaide.edu.au

Xin Pan
MBRC/HMRI, NSW
xin.pan@uon.edu.au

Tammy Pang
University of Melbourne, VIC
p.pang2@pgrad.unimelb.edu.au

Vijay Panicker
Sir Charles Gairdner Hospital, WA
Vijay.Panicker@health.wa.gov.au

Sarah Pattison
Austin Health, VIC
pattisonsarah@hotmail.com

David Pattison
Royal Melbourne Hospital, VIC
david.pattison@mh.org.au

Jonathan Paul
University of Newcastle, NSW
Jonathan.Paul@newcastle.edu.au

Ryan Paul
Waikato DHB,
paulr@waikatodhb.govt.nz

Sarah Paule
Prince Henrys Institute of Medical Research, Vic
sarah.paule@princehenrys.org

Michael Pearen
Institute for Molecular Bioscience, QLD
m.pearen@uq.edu.au

Heidi Peason
Servier, VIC
heidi.pearson@au.netgrs.com

Elise Pelzer
Institute of Health and Biomedical Innovation, QLD
e.pelzer@qut.edu.au

Nimalie Perera
Royal Prince Alfred Hospital, NSW
nimalie.perera@email.cs.nsw.gov.au

Viv Perry
QSML & RRRC, Qld
viv@qsml.com.au

Carolyn Petersons
Southern Adelaide Diabetes and Endocrine Services, SA
carolynpetersons@yahoo.com.au

Annalise Pham
Canberra Hospital, ACT
apham@internode.on.net

David Phillips
La Trobe University, Vic
david.phillips@latrobe.edu.au

Mike Pickford
ASN Events, Vic
mp@asnevents.net.au

Mario Pierides
Abacus ALS, QLD
m.pierides@abacus-als.com

Eduardo Pimenta
University of Queensland Medical School, QLD
e.pimenta@uq.edu.au

Agata Piotrowicz
John Hunter Hospital, NSW
agatapiotrowicz@yahoo.com

Walter Plehwe
The Epworth Centre, Vic

plehwe@optusnet.com.au

Stacey Plug
University of Western Australia, WA
plugs01@student.uwa.edu.au

Connie Poon
The University of Sydney, NSW
conniep@anatomy.usyd.edu.au

Solomon Posen
University of Sydney, NSW
solposen@mail.usyd.edu.au

Richard Prince
University of Western Australia, WA
richard.prince@uwa.edu.au

Kirsty Pringle
University of Newcastle, NSW
kirsty.pringle@newcastle.edu.au

Tuan Quach
John Hunter Hospital, NSW
tuan.quach@newcastle.edu.au

M RAFFOUL
UPTODATE, NSW
mraffoul@uptodate.com

Rohit Rajagopal
Campbelltown Hospital, NSW
rohit1@optusnet.com.au

Kate Redgrove
The University of Newcastle, NSW
Kate.Redgrove@uon.edu.au

Andrew Reid
The University of Newcastle, NSW
Andrew.T.Reid@newcastle.edu.au

Renee ReijoPiera
Stanford University, CA
reneer@stanford.edu

Marilyn Renfree
University of Melbourne, VIC
m.renfree@unimelb.edu.au

Debra Renouf
Southern Health, VIC
debra_renouf@hotmail.com

David Roberts
Queensland Health, QLD
david_roberts@health.qld.gov.au

Claire Roberts
University of Adelaide, SA
claire.roberts@adelaide.edu.au

Sarah Robertson
University of Adelaide, SA
sarah.robertson@adelaide.edu.au

Ray Rodgers
University of Adelaide, SA
ray.rodgers@adelaide.edu.au

Peter Rogers
Monash University, VIC
peter.rogers@med.monash.edu.au

Shaun Roman
University of Newcastle, NSW
shaun.roman@newcastle.edu.au

Mary Rookledge
CSIRO, NSW
mary.rookledge@csiro.au

Glynis Ross
Royal Prince Alfred Hospital/Bankstown-Lidcombe Hospital, NSW
gpross@bigpond.net.au

Alexina Ross-Smith
Medtronic, NSW
alexina.ross-smith@medtronic.com

Janet Rowan
National Women's Health, Auk
jrowan@internet.co.nz

Nirupa Sachithanandan
St Vincent's Hospital and St Vincent's Institute, VIC
nsachithanandan@hotmail.com

Joanna Sader
Medtronic, NSW
joanna.sader@medtronic.com

Zeliha Sahin
Akdeniz University, Akd
sahin_zelish@hotmail.com

Anuradha Sakthivel
Royal Women's Hospital, VIC
anu_velu@yahoo.com

Christy Sankoorikal
John Hunter Hospital, NSW
christypauls@gmail.com

Mai Sarraj
Prince Henry's Institute, Vic
mai.sarraj@princehenrys.org

Sabine Schmoelzl
CSIRO, NSW
sabine.schmoelzl@csiro.au

Katherine Scott
Gold Coast Hospital, QLD
kathscott76@yahoo.com.au

Chris Scott
Charles Sturt University, NSW
chscott@csu.edu.au

Gordon Senator
Griffith University, QLD
gordbren18@gmail.com

Brian Setchell
University of Adelaide, SA
brian.setchell@adelaide.edu.au

Erin Sharp
Merck Serono Australia, NSW
erin.sharp@merckserono.net

Rosemary Sheehy
SSWAHS, NSW
rosemary@email.cs.nsw.gov.au

Jimmy Shen
Southern Health, VIC
jimmyshenzl@yahoo.com

Vasant Shenoy
Mater Health Services , QLD
dr.vshenoy@gmail.com

Roger Short
The University of Melbourne, VIC
r.short@unimelb.edu.au

Nicki Sidwell
Novartis Pharmaceuticals, NSW
nicki.sidwell@novartis.com

Ie-Wen Sim
St Vincent's Health, Vic
iewen@hotmail.com

Lisa Simmons
Royal Prince Alfred Hospital, NSW
lsimmons@med.usyd.edu.au

Stephanie Simonds
Monash University, Vic
Stephanie.simonds@monash.edu

Harmeet Singh
Prince Henry's Institute of Medical Research, VIC
harmeet.singh@princehenrys.org

Jeremy Smith
Monash University, VIC
jeremy.smith@monash.edu

Roger Smith
Hunter Medical Research Institute, NSW
Roger.Smith@newcastle.edu.au

A SMITH
UPTODATE, NSW
asmith@uptodate.com

Rob Smits
Rivalea Australia, NSW
rsmits@rivalea.com.au

Alexander Sobinoff
The University of Newcastle, NSW
Alexander.Sobinoff@newcastle.edu.au

Stefan Sonderegger
Prince Henry's Institute of Medical Research, VIC
stefan.sonderegger@princehenrys.org

Steve Soule
Christchurch Hospital, NZ
steven.soule@cdhb.govt.nz

Justin St. John
Monash Institute of Medical Research, VIC
justin.StJohn@med.monash.edu.au

Kaye Stenvers
Prince Henry's Institute, VIC
kaye.stenvers@princehenrys.org

Victoria Stott
Dunedin Hospital, Otago
vicstott@hotmail.com

Michael Stowasser
University of Qld School of Medicine, Qld
m.stowasser@uq.edu.au

Stephen Stranks
Southern Adelaide Diabetes and Endocrine Services, S.A.
steve.stranks@health.sa.gov.au

Jessica Stringer
The University of Melbourne, Vic
j.stringer@pgrad.unimelb.edu.au

Bronwyn Stuckey
Keogh Institute for Medical Research, WA
bstuckey@cyllene.uwa.edu.au

George Stylianou
MSD, NSW
george.stylianou@merck.com

Siti Aishah Sulaiman
UNI OF ADELAIDE, SA
siti.sulaiman@adelaide.edu.au

Shyam Sunder
Nambour General hospital, QLD
sunder88@gmail.com

Emershia Suthaharan
Cairns Base Hospital, QLD
nilanjanasutha@yahoo.com

Jessie Sutherland
The University of Newcastle, NSW
Jessie.Sutherland@uon.edu.au

Shane Sykes
Mothers and Babies Research Centre, Hunter Medical Research Institute, John Hunter Hospital, NSW
c3045176@uon.edu.au

Lyndal Tacon
Kolling Institute of Medical Research, NSW
lyndal.tacon@sydney.edu.au

Jonathan Taft
Private Practice, Vic
jtaft@bigpond.net.au

Simone Tannock
The University of Newcastle, NSW
Simone.Tannock@newcastle.edu.au

Helena Teede
Southern Health, Vic
jhf.diabetes@med.monash.edu.au

Amanda Terry
Tennyson Centre, SA
amanda.terry@internode.on.net

Sabina Testa
University of Sydney, NSW
saby82@hotmail.it

Edward Thomas
SciGen(Australia) Pty Ltd, NSW
ethomas@scigen.com.au

Anna Thomas
Private Practice, QLD
annalthomas@bigpond.com.au

Michael Thompson
University of Sydney, NSW
mike.thompson@bio.usyd.edu.au

Jeremy Thompson
The University of Adelaide, SA
jeremy.thompson@adelaide.edu.au

Tilenka Thynne
Royal Adelaide Hospital, SA
tilenka.thynne@gmail.com

Wayne Tilley
UNIVERSITY OF ADELAIDE, SA
marie.pickering@adelaide.edu.au

Sarah To
Prince Henry, VIC
sarah.to@princehenrys.org

Roxanne Toivanen
Monash University, VIC
roxanne.toivanen@med.monash.edu.au

Jorge Tolosa
The University of Newcastle, NSW
jorge.tolosagonzalez@uon.edu.au

Stephen Tong
Monash Medical Centre, Vic
stephentong1@gmail.com

Carol Tong
Monash Medical Centre, Vic
carolhllee@hotmail.com

Duncan Topliss
The Alfred, Vic
duncan.topliss@monash.edu

David Torpy
Royal Adelaide Hospital, SA
david.torpy@health.sa.gov.au

Huy Tran
HUNTER AREA PATHOLOGY SERVICE, NSW
huy.tran@hnehealth.nsw.gov.au

Jane Tran
Western Hospital, VIC
janet_ran@hotmail.com

Melanie Tran
University of Melbourne, VIC
m.tran6@pgrad.unimelb.edu.au

Khoa Truong
The University of Melbourne, VIC
k.truong2@pgrad.unimelb.edu.au

Venessa Tsang
Kolling Institute, NSW
venessa_t@hotmail.com

Anne Turner
Deakin University, VIC
anne.turner@deakin.edu.au

Tamara Varcoe
University of Adelaide, SA
tamara.varcoe@adelaide.edu.au

Shilpa Verma
Box Hill Hospital, VIC
shilpaverma@hotmail.com

Chez Viall
University of Auckland, Auckl
c.viall@auckland.ac.nz

Prathima Vijayakumar
Sapphire Bioscience Pty. Ltd., NSW
prathimav@sapphirebioscience.com

Thuy Vu
Austin Health, Vic
thuystar@hotmail.com

Brendan Waddell
The University of Western Australia, WA
brendan.waddell@uwa.edu.au

Petra Wale
University of Melbourne, VIC
pwale@pgrad.unimelb.edu.au

David Walker
Medtronic, NSW
david.w.walker@medtronic.com

Yao Wang
PHI, VIC
yao.wang@princehenrys.org

Yu (Eric) Wang
Mothers and Babies Research Centre HMRI JHH, NSW
doohma@hotmail.com

Mike Waters
University of Queensland, Qld
m.waters@uq.edu.au

Laura Watson
Adelaide University, SA
laura.n.watson@adelaide.edu.au

Toni Welsh
University of Newcastle, NSW
toni.welsh@newcastle.edu.au

Michaela Wharfe
The University of Western Australia, WA
20261643@student.uwa.edu.au

Danielle White
ASN Events, VIC
dw@asnevents.net.au

Sarah Wilkinson
Monash University, VIC
wilkinson830@gmail.com

Paul Williams
University of Sydney, NSW
pwlms@med.usyd.edu.au

Kathryn Williams
St Vincents Hospital, NSW
kath_williams@hotmail.com

Errol Wilmshurst
Northern Sydney Endocrine Centre, NSW
ejwilmshurst@bigpond.com

Wendy Winnall
Monash Insititute of Medical Research, VIC
Wendy.Winnall@monash.edu

Mary Wlodek
University of Melbourne, VIC
m.wlodek@unimelb.edu.au

Jennifer Wong
Southern Health, VIC
jennifer.wong@monash.edu

Rosemary Wong
Western Hospital, Vic
wongrose@bigpond.net.au

Tricia Wong
Peninsula Health, VIC
twcwong@hotmail.com

Tang Wong
Prince of Wales Hospital, NSW
drtwong@optusnet.com.au

Rosie Worsley
The Geelong Hospital, VIC
rosie.worsley@gmail.com

Paul Wraight
The Royal Melbourne Hospital, Vic
paul.wraight@mh.org.au

Hui Wu
Monash Institute of Medical Research, Monash University, vic
hui.wu@monash.edu

Bu Yeap
University of Western Australia, WA
byeap@cyllene.uwa.edu.au

Ming li Yee
Southern Health, Vic
mingli2980@yahoo.com

Christine Yeo
The University of Melbourne, Vic
cxyeo@unimelb.edu.au

Neil Youngson
QIMR, QLD
neil.youngson@qimr.edu.au

Marina Zaitseva
Monash University, Vic
marina.zaitseva@monash.edu

Tamas Zakar
Mothers and Babies Research Centre, NSW
tamas.zakar@newcastle.edu.au

Deirdre Zander-Fox
Repromed, SA
dzander@repromed.com.au

Jane Zhang
Campbelltown Hospital, NSW
janezhang1@hotmail.com

NOTES

NOTES

NOTES

The conference acknowledges the sponsorship of sanofi aventis
Because health matters

NOTES

The conference acknowledges the sponsorship of sanofi aventis
Because health matters