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ESA OFFICE BEARERS 2009

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PAST ESA OFFICE BEARERS 1958-2008

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
1992-94	D.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	V. Clifton	D. Phillips

SOCIETY SECRETARIAT - ENDOCRINE SOCIETY OF AUSTRALIA

Ivone Johnson
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SERVIER

FUTURE MEETINGS

ESA Seminar
30th April – 2nd May 2010
Novotel Woollongong
www.esaseminar.org.au

ESA Clinical Weekend
27th August – 29th August 2010
NSW
www.esaclinicalweekend.org.au

Combined ESA/SRB Annual Scientific Meeting
29th August – 1st September 2010
Sydney Convention Centre
www.esa-srb.org.au

KEITH HARRISON MEMORIAL LECTURERS

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

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

ESA CSL BIOTHERAPIES 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

ESA / IPSEN INTERNATIONAL TRAVEL GRANT

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

SERVIER AWARD

The Servier Award is awarded for the best published work in the previous year by a member of the Society within 5 years of award of higher degree.

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

HONORARY LIFE MEMBERS

Dr Robert Baxter
Dr A.W. Blackshaw
Dr H.D. Breidahl
Prof James B Brown
Prof Henry G Burger
Dr R.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 C J Eastman AM
Dr K.A. Ferguson
Prof John W Funder
Prof Peter Fuller
Prof R.D. Gordon
Dr Ian B Hales
Prof David Handelsman
Dr Philip Harding
Prof Basil Hetzel
Dr Brian Hirschfeld
Dr Ivan G Jarrett
Assoc Prof. Stephen Judd
Prof Richard G Larkins
Prof Leslie Lazarus
Dr T.B. Lynch
Prof Ian McDonald
Prof T John Martin
Dr Len Martin
Dr F.I.R. Martin
Dr Ian C A Martin
Dr Roger Melick
Prof Solomon Posen
Prof Marilyn Renfree
Prof Gail Risbridger
Prof T.J. Robinson
Prof Rodney Shearman
Prof Alfred W Steinbeck
Prof Jim Stockigt
Prof R. Strang
Prof Pincus Taft
Dr Ian D Thomas
Prof Duncan Topliss
Prof Prof Victor Trikojus
Emeritus Prof John R Turtle
Prof R. Vines
Dr A.L. Wallace
Prof Norman Wettenhall
Prof F. H Wilson
Prof Marelyn Wintour-Coghlan
Dr K.N. Wynne

CONFERENCE ORGANISING COMMITTEES

The Local Organising Committee
Convenor: Nicolette Hodyl (ESA)
Ravinder Anand-Ivell (ESA), Hannah Brown (SRB), Kathy Gatford (ESA), Rob Gilchrist (SRB),Jui Ho (ESA), Wendy Ingman (SRB), Helen MacLean (ESA), Annette Osei-Kumah (ESA), Darryl Russell (SRB), Michael Stark (ESA)

ESA Program Organising Committee
Helen MacLean (chair), Carolyn Allan, Greg Anderson, Mathis Grossmann, Warrick Inder, Brian Oldfield, Vince Russo
The Program Committee thanks those members who assisted in reviewing the abstracts.

Conference Secretariat
ASN Events Pty Ltd
3056 Frankston-Flinders Road, (PO Box 200)
BALNARRING VIC 3926
Phone: 03 5983 2400 Fax: 03 5983 2223
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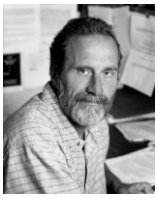
INVITED PLENARY SPEAKER PROFILES 2009

HARRISON LECTURER



John Cidlowski - John A. Cidlowski is Chief of the Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, NIH, in North Carolina. He received his PhD in endocrinology from the Medical College of Georgia, and after a post-doctoral fellowship in molecular endocrinology at Dartmouth Medical School, he joined the faculty at the University of Vermont College of Medicine. He moved to the University of North Carolina, Chapel Hill in 1982, where he served as Professor of Physiology and Biochemistry from 1990, before joining the NIEHS in 1995. Dr Cidlowski was Editor in Chief of Molecular Endocrinology from 2004 to 2008, and is on the editorial board of other journals including Cell Death and Differentiation and Journal of Steroid Biochemistry. He has received a number of awards during his career, including plenary lectures at the International Congress of Hormonal Steroids, and most recently the Edwin B. Astwood Award from the US Endocrine Society in 2008. The major areas of Dr Cidlowski's research interest are glucocorticoid receptors and their actions on the inflammatory response, and regulation of apoptosis in normal and neoplastic cells. Dr Cidlowski has published more than 270 papers in leading biomedical journals, as well as several book chapters. His contributions include the first paper to show that proteolysis is a fundamental component of apoptosis, identification of the A and B forms of the human glucocorticoid receptor, demonstrating that steroid receptors are regulated during the cell cycle and the first demonstration that phosphorylation affects the transcriptional activity of glucocorticoid receptors.

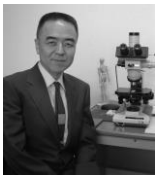
TAFT LECTURER

Karen Miller - Karen K. Miller, MD is a faculty member in the Neuroendocrine Unit at the Massachusetts General Hospital and at Harvard Medical School and the Director of the Neuroendocrine Research Program in Women's Health at Massachusetts General Hospital (MGH). She is a nationally known clinical researcher on the effects of undernutrition and neuroendocrine dysregulation, including androgen and growth hormone (GH) deficiency, on body composition, cardiovascular risk and other endpoints in women. Her work demonstrating improvement an increase in bone density and muscle mass and an improvement in depression severity in women with hypopituitarism receiving physiologic testosterone replacement, won The Endocrine Society International Award for Excellence in Published Clinical Research in The Journal of Clinical Endocrinology and Metabolism in 2006. In addition, she has served as the Chair of the Mentorship Committee for Women in Endocrinology, and as a member of The Endocrine Society Androgen Deficiency in Women Guidelines Committee, the American Association of Clinical Endocrinology (AACE) Acromegaly Management Guidelines Revision Task Force, the editorial board of the Journal of Clinical Endocrinology and Metabolism, and The Endocrine Society Research Committee, among other organizations.

NZSE / ESA NANCY SIRETT LECTURER

Peter Gluckman - Prof Peter D Gluckman is Professor of Paediatric and Perinatal Biology, Director of the Liggins Institute for Medical Research and Director of the National Research Centre for Growth and Development at the University of Auckland. Prof Gluckman trained in paediatrics and endocrinology at the Universities of Otago and Auckland and the University of California, San Francisco. He returned to NZ to establish a group in perinatal physiology, and in 1998 was appointed a chair in Paediatric and Perinatal Biology and became chairman of the Dept of Paediatrics. From 1992 – 2001 he was executive Dean of the Faculty of Medicine and Health Sciences. In 2001 Prof Gluckman was appointed foundation director of the Liggins Institute for Medical Research of the University of Auckland. His research encompasses the regulation of fetal and postnatal growth, developmental epigenetics, the developmental origins of metabolic disease, and the evolutionary-developmental biology-medical interface. He has published over 450 refereed papers, 150 reviews and edited several books and authors both technical and popular science books. He is inventor on over 25 families of patent. Prof Gluckman was awarded a DSc (Auckland), elected a fellowship of the Royal Society of NZ, conferred a Companion of the NZ Order of Merit in 1997 and elevated to Distinguished Companion in 2008. He was appointed a foundation University Distinguished Professor by the University of Auckland in 2001. He serves on numerous Editorial Boards and is former President of the International Society for the Developmental Origins of Health and Disease. In 2001 he was awarded New Zealand's highest scientific honour, the Rutherford Medal. He was the NZ Herald's New Zealander of the Year in 2005. He was elected a Fellow of the Royal Society (London) in 2001, a foreign member of the Institute of Medicine of the National Academy of Sciences (USA) (2004) and a Fellow of the Academy of Medical Sciences UK (2006). In 2007 while continuing his role in Auckland, Prof Gluckman was appointed Programme Director for Growth, Development and Metabolism at the Singapore Institute of Clinical Sciences. He also holds honorary chairs at the National University of Singapore and the University of Southampton.

JAPAN / AUSTRALIA LECTURER



Hironobu Sasano - Hironobu Sasano is Professor of the Department of Pathology, Tohoku University School of Medicine, and Director of the Department of Pathology, Tohoku University Hospital. He obtained his MD from Tohoku University School of Medicine, Sendai, Japan, and trained as a Fulbright exchange research and clinical fellow in the Division of Pediatric Endocrinology, at The New York Hospital - Cornell Medical Center. After residency training in anatomic pathology at The George Washington University Hospital, Washington DC, he returned to the Tohoku University School of Medicine in 1989, where he has been Professor and Director since 1998. Prof Sasano's research interest is steroid biosynthesis, metabolism and actions, with a focus on intracrine actions in cancer, and he has published nearly 400 peer-reviewed papers. Prof Sasano serves on the executive of a number of professional societies including the Japan Endocrine Society, the Japanese Pathology Society and the Society of Cardiovascular Endocrinology and Metabolism. His editorial board memberships include Molecular and Cellular Endocrinology, Molecular Oncology, and Journal of Steroid Biochemistry and Molecular Biology (associate editor).

PLENARY LECTURER

Joseph Verbalis - Joseph G. Verbalis, MD, graduated from Princeton University with an AB in chemistry, and received an MD from the University of Pittsburgh in 1975. After completing his residency training at the Hospital of the University of Pennsylvania in Philadelphia, and fellowship training in endocrinology and metabolism at the University of Pittsburgh, Dr Verbalis was a faculty member at the University of Pittsburgh from 1980 to 1995, where he rose to the position of tenured Professor of Medicine. He served as the Chief of the Division of Endocrinology and Metabolism at Georgetown University in Washington, DC, and then as the Interim Chair of the Department of Medicine. Dr Verbalis is currently a Professor of Medicine and Physiology, Chief of the Division of Endocrinology and Metabolism, Program Director of the General Clinical Research Center, and Clinical Director of the Center for the Study of Sex Differences in Health, Aging and Disease at Georgetown University. Dr Verbalis has published more than 250 journal articles and book chapters related to the neuroendocrine regulation of the hormones vasopressin and oxytocin, and disorders of body fluid homeostasis. His recent research has concentrated on mechanisms underlying adaptation to hyponatremia, renal escape from vasopressin, osmotic regulation of hypothalamic gene expression, sex differences in physiology and pathophysiology, exercise-associated hyponatremia and clinical use of vasopressin receptor antagonists. He authors the chapters on vasopressin and water metabolism in major textbooks of endocrinology (Williams Textbook of Endocrinology; Principles and Practice of Endocrinology and Metabolism), nephrology (Brenner & Rector's The Kidney; Diseases of the Kidney and Urinary Tract), and neuroscience (Fundamental Neuroscience). Invited lectureships include the Presidential Lecture of the American College of Sports Medicine, the Robert W. Schrier lectureship at the American Society of Nephrology, and the Berthold Medal lectureship of the German Endocrine Society. Dr Verbalis is currently an editorial board member for Frontiers in Neuroendocrinology and the Postgraduate Medicine.

ESA / ADS LECTURER

Mark Febbraio - Professor Mark Febbraio is a Principal Research Fellow of the NHMRC, is the head of the Cellular and Molecular Metabolism Laboratory and Director of Basic Science in the Division of Metabolism and Obesity at the Baker IDI Heart & Diabetes Institute. He currently oversees approximately 30 staff and students. His laboratory is focussed on understanding cellular and molecular mechanisms associated with lipid-induced inflammation and insulin resistance. He has made the vital discovery that muscles produce and secrete cytokines that have biological reactivity. This has lead to the very important discovery that IL-6 and other IL-6 family cytokines can be used as anti-obesogenic compounds and this has culminated in the publications in journals such as *Nature Medicine, Journal of Clinical Investigation, Cell Metabolism, Proc Natl Acad Sci USA, Diabetes, Journal of Biological Chemistry and FASEB Journal*. Professor Febbraio is regularly invited to overseas scientific symposia including *Keystone Symposia and The American Diabetes Association Annual Scientific Meeting*. He has won prizes at international, national and institutional levels including the A K McIntyre Prize for significant contributions to Australian Physiological Science (1999), and the Colin I Johnson Lectureship by the High Blood Pressure Research Council of Australia. He is on the Editorial board of Diabetes, *The American Journal of Physiology Endocrinology & Metabolism, Exercise Immunology Reviews and Journal of Applied Physiology*.

New KwikPen has landed

KwikPen – A new insulin carrier for Australians with diabetes

Announcing KwikPen – a new prefilled insulin pen from Eli Lilly. Small, lightweight (weighs 31 grams) and easy to use.[1,2] Ideal for on-the-go patients of all ages.[1,3]
KwikPen. The Kwik way.

PBS LISTED 1 JULY[4]

PBS Information: General benefit. Treatment for Diabetes.
Refer to PBS Schedule for full PBS listing information for each product.

Humalog mix25™ new KwikPen™
25% insulin lispro (rDNA origin) injection
75% insulin lispro protamine suspension

HUMALOG,® HUMALOG MIX25,® HUMALOG MIX50® MINIMUM PRODUCT INFORMATION. PLEASE REVIEW APPROVED PRODUCT INFORMATION BEFORE PRESCRIBING. FULL PI IS AVAILABLE FROM ELI LILLY. **Approved Indication:** Treatment of diabetes mellitus. **Contraindications:** Hypoglycaemia; hypersensitivity to insulin lispro or one of its excipients; intravenous administration. **Precautions:** Any change of insulin or human insulin analogue should be made under medical supervision; loss of warning symptoms of hypoglycaemia; adjust dose for changes in exercise, diet and illness; should not be mixed with other insulins. **Adverse Reactions:** Hypoglycaemia, allergic reactions and lipodystrophy. **Dosage:** As determined by physician; subcutaneous injection; before meals (15 minutes). **PBS dispensed price:** Humalog KwikPen, Humalog Mix25 KwikPen, Humalog Mix50 KwikPen $263.79 (5x5x3mL); Humalog vial $158.84 (5x10mL). Refer to full PI for complete dosage information. Please review full PI before prescribing. **Full PI is available from Eli Lilly Australia Pty. Limited. 112 Wharf Road, WEST RYDE NSW 2114. Based on PI last amended 14 May 2009. References 1.** Brown AW. (2008) *Clinical Diabetes* 26(2):66-71. **2.** Ignaut DA, et al. (2008) *J Diabetes Sci Technol* 2(3):533-5. **3.** Fowler MJ. (2000) *Clinical Diabetes* 26(3):130-133. **4.** Australian Government, Department of Health and Ageing. PBS http://www.pbs.gov.au/html/home

Lilly

INFORMATION FOR DELEGATES & PRESENTERS

Venue
Adelaide Convention Centre
North Terrace
Adelaide SA 5000, Australia
Ph: +61 8 8212 4099

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 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 in Foyer H, which is outside the trade and poster display area. All breaks are taken in this space. The plenary lectures and concurrent sessions are mainly held in the lecture theatre complex separated by a small walk way from the exhibition building. Please see venue map on next page.

Organiser's Office and Registration Desk
The organiser's office and registration desk will be located immediately outside the exhibition entrance in Foyer H. The registration desk will be open on Sunday 23rd August from 12:00 PM to 6:00 PM and on Monday 24th and Tuesday 24th 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 (Meeting Room 3) 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.

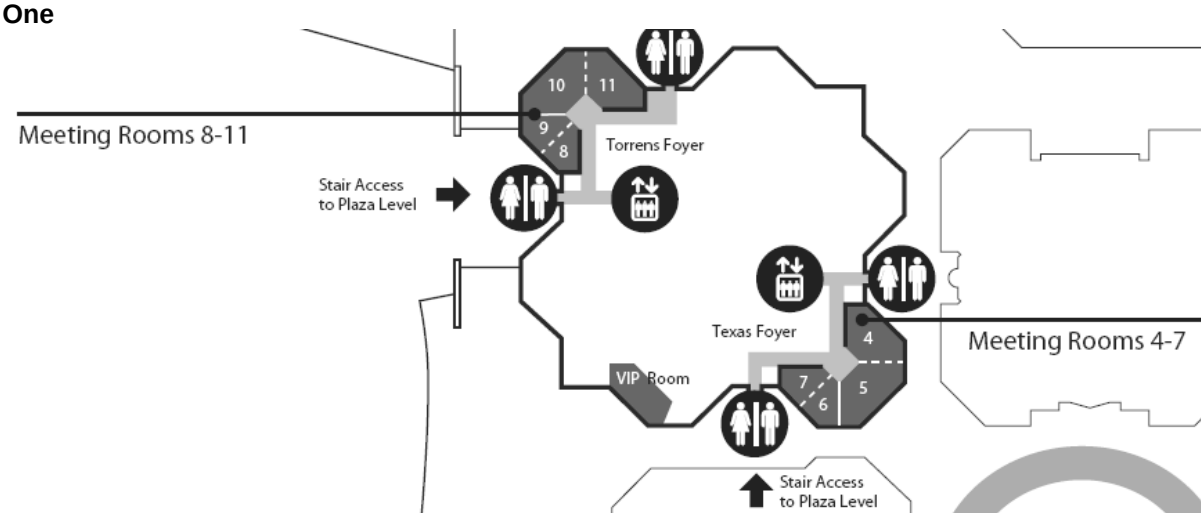
Name Tags
Delegates are required to wear their name tags to all scientific and catered sessions. Uniformed security is in attendance on the doors of the exhibition area and name tags are required to gain access.
Delegates should note that within their name tag pouch are any specific function tickets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

Poster Viewing

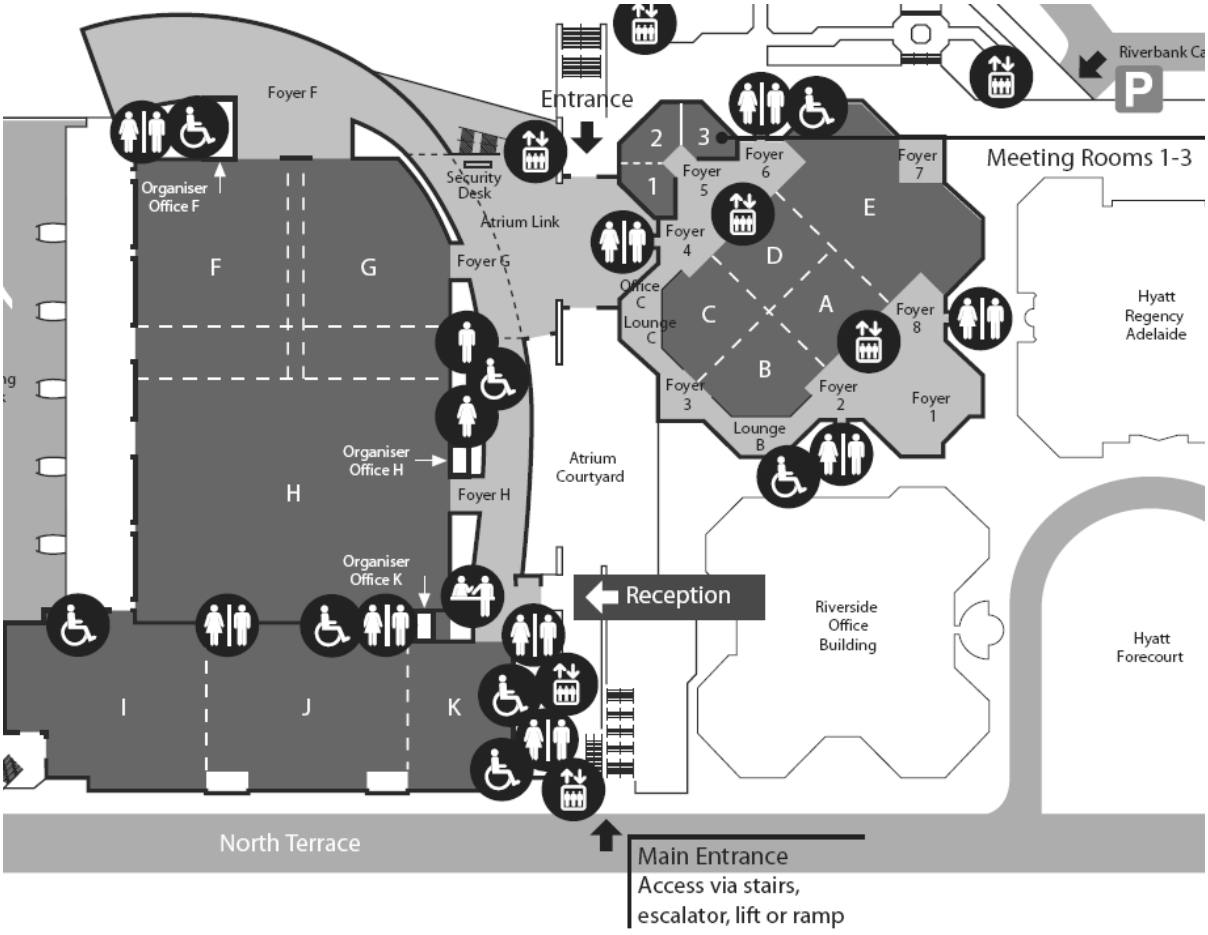
Delegates with posters can find the correct position for their poster by finding the appropriate abstract number on the display panels. The panels are set up in the exhibition hall. The program provides your abstract number which is how you find you placement position. Posters can remain on display all of Monday and Tuesday and must be removed by morning tea on Wednesday. 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 Novartis Oncology*
There will be an internet café provided for delegates for the duration of the meeting. The café is located on the right hand side of the exhibition hall.

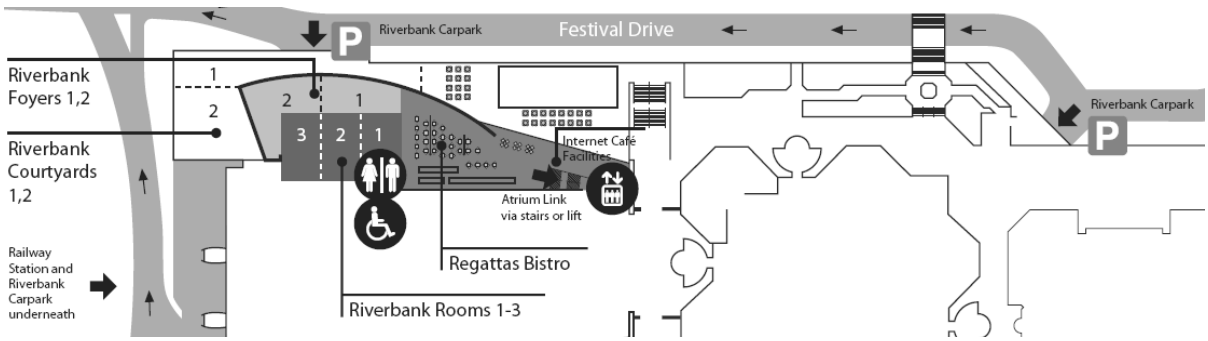
Level One



Plaza Level

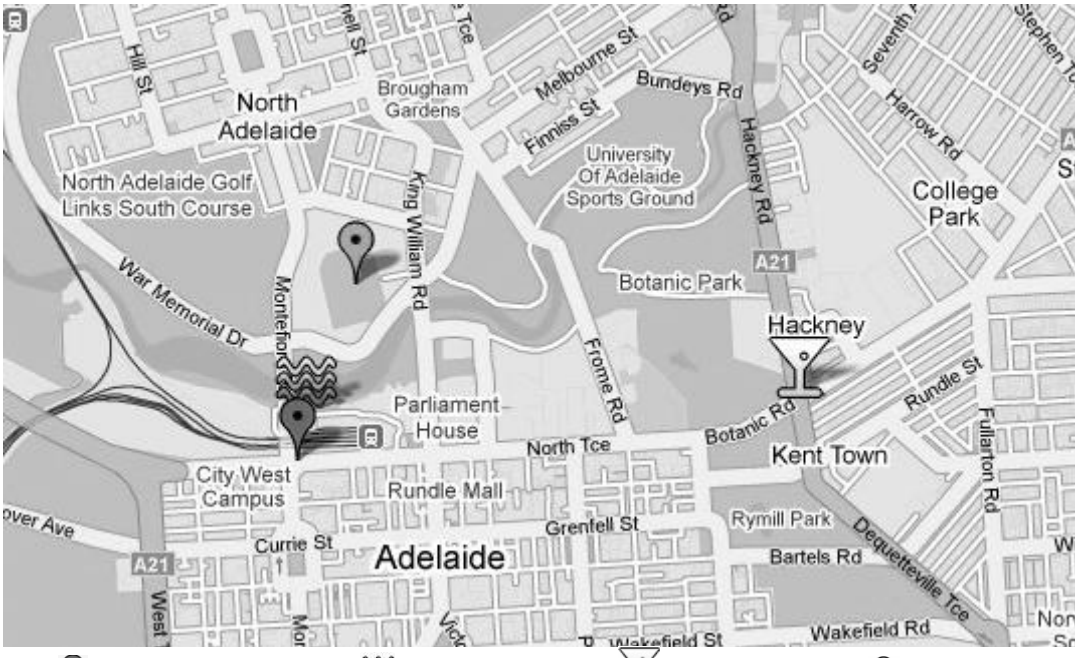


Riverbank Level



Social Functions

- The **Welcome Function** is at the Adelaide Convention Centre on the Sunday evening from 5:30pm. Light refreshments and drinks will be served and the function is complimentary for all registration types. The function will take place on Foyer F.
- The SRB are celebrating their **40th birthday** and are holding a special celebratory dinner at the Adelaide Oval in the Gil Langley room. The dinner will be on Sunday 23rd August from 7pm for a 7:30pm start, following the Welcome Function at the Convention Centre. This is a ticketed function and tickets are required to attend the dinner. Your tickets can be found in your delegate materials; please visit the registration desk if you wish to purchase extra tickets (subject to availability). To get to the Adelaide Oval exit the Convention Centre towards the Torrens River and turn right. Walk along the river and cross at the first bridge, at King William Road. Turn left down War Memorial Drive, which is the first street after the bridge. About 100 metres down the road is a car park on the right, walk through it to the Phil Ridings gates. Through the gate is an entrance underneath the Sir Donald Bradman Stand sign. Enter and take the elevator to the 3rd floor. SRB LOC members will also be walking to the SRB Function dinner from the conference centre following the Welcome Reception. Anyone also wishing to walk can join these groups at the Welcome reception. Groups will leave from ~6:50 – 7:10pm and walk to the Adelaide Oval.
- The Monday night **Student Function** is being held at Adelaide Rowing Club, Festival Drive, Torrens Lake, Adelaide. 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 function begins at 7:00pm and dress is neat casual. To get to the Adelaide Rowing Club, simply walk out of the Convention Centre facing the Torrens River, walk down to the riverside and turn left (West). The Adelaide Rowing club is one of few boat sheds along the river before you reach the Morphett St bridge. **This is a ticketed function** and they must be purchased in advance.
- The **Conference Dinner** will be held on Tuesday evening at the National Wine Centre of Australia. 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. The Wine Centre is located on the corner of Botanic and Hackney Roads, Adelaide. Buses will not be provided to transport delegates to the dinner venue, however please see below travel directions:
  - It will take approximately 15 - 20 minutes to walk from the Convention Centre to the Wine Centre down North Terrace.
  - There is a free bus called the 99C that travels along North Terrace and drops off at Royal Adelaide Hospital. The last bus will service at 6.05pm.
  - After 6pm the M44 bus runs every 15 minutes and will pick up from the F2 stop on North Terrace and King William Road. The bus will then drop off at stop 2 on Hackney Road, close to the Wine Centre. The cost for this bus is $1.20 for concession and $2.50 for regular fares.
  - If travelling by car - A passenger set down area is available on Hackney Road. Short term parking is available along Hackney Road and Plane Tree Drive, and car parks are located on Frome Road, Rundle Street and North Terrace. Please note on-site parking facilities are limited.
  - Taxis will cost approximately $10 AUD to get to the National Wine Centre from the Adelaide Convention Centre.



Adelaide Convention Centre Adelaide Rowing Club National Wine Centre Adelaide Oval

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.

Tuesday 25th August, 2009

AMGEN AUSTRALIA - *Molecular signalling pathways as novel therapeutic targets in bone health*
7:30 AM – 8:20 AM Meeting Room 10
Chair: Peter Ebeling, *Professor of Medicine, Head of Endocrinology, Department of Medicine, Royal Melbourne Hospital/Western Hospital, University of Melbourne*
Session 1: "Rank ligand and related mechanisms in bone regulation"
Speaker: Jack Martin, *Emeritus Professor of Medicine, Bone, Joint & Cancer Unit, St Vincent's Institute of Medical Research, VIC*
Session 2: "The calcium sensing receptor as a therapeutic target in primary hyperparathyroidism: the role of calcimimetics"
Speaker: Roderick Clifton-Bligh, *Senior Lecturer, Medicine, Northern Clinical School, Kolling Institute of Medical Research, NSW*

Wednesday 26th August, 2009

BREAKFAST CAREER WORKSHOPS (ESA / SRB / ADS)
The Career Development Workshops are a must for any junior members, and are open to all ESA, SRB and ADS meeting registrants. Two concurrent workshops will be run, one for clinicians and the other for basic scientists. The sessions are from 7.00 am to 8.30 am Wednesday August 26th, and will include a free breakfast. Each session comprises three twenty-minute presentations, followed by half an hour of general Q&A. The sessions are informal, and questions are not limited to the topics being presented, but can cover any issue you might have related to your career in endocrine research or endocrinology.

ESA BREAKFAST CAREER WORKSHOP FOR JUNIOR SCIENTISTS
7:00 AM - 8:25 AM Meeting Rooms 1 & 2

7:00am **Vicki Clifton**
Scientific career development for clinicians and basic scientists: A balance act between work, research, family and friends

7:20am **Tom Kay**
Keys to delivering a polished presentation

7:40am **Kate Loveland**
Managing a research lab: Making your career fun and productive

8:00am Q & A

ESA BREAKFAST CAREER WORKSHOP FOR JUNIOR CLINICIANS
7:00 AM - 8:25 AM Hall D

7:00am **Shaun McGrath**
What to do in your third year of Endocrine training *abs#042*

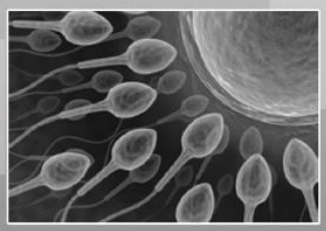
7:20am **Rory Clifton-Bligh**
Should you do a research higher degree? *abs#043*

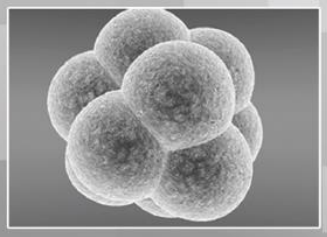
7:40am **Harvey Newnham**
Issues involved in private practice *abs#044*

8:00am Q & A

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 takes a different approach to animal welfare, addressing the causes and as well as the consequences of pet overpopulation. Through innovative strategies and community partnerships, Found Animals is working to develop sustainable, scalable animal welfare business models which minimize shelter euthanasia.

Found Animals is focused on creating innovative, largely self-sustaining programs dedicated to three initiatives:

- Companion animal sterilization
- Resources for pet owners
- Adoption

Sunday, 23rd August 2009

SRB Workshop: Graeme Martin - Science Communication and Writing

2:00 PM - 3:30 PM Hall D

SRB Symposium: Pluripotency and Stem Cells in Reproduction

3:30 PM - 5:00 PM Hall D

Chairs: Chris O'Neill and Hugh Morgan

The conference acknowledges the support of Novo Nordisk

3:30pm **Mark Nottle**
Isolation and characterisation of porcine ES cells *abs#001*

4:00pm **Patrick Western**
Regulation of pluripotency and cell cycle in fetal germ cells *abs#002*

4:30pm **Caroline Gargett**
Endometrium: Cinderella tissue in a stem cell world *abs#003*

RCRH MCR Award

5:00 PM - 5:30 PM Hall D

Rob Gilchrist
The mammalian oocyte: from bench to clinic *abs#065*

ESA/SRB Welcome Function

5:30 PM - 7:00 PM Foyer F

The conference acknowledges the support of Novo Nordisk

Monday, 24th August 2009

ESA Taft Plenary Lecture

8:30 AM - 9:30 AM — Hall B

Chair: Helena Teede

Karen Miller

Endocrinology of anorexia nervosa *abs#004*

SRB Orals: Stem Cells

8:30 AM - 10:00 AM — Meeting Rooms 10 & 11

Chairs: Mark Nottle and Caroline Gargett

8:30am **Louie Ye**
In Vivo Differentiation of Human Embryonic Stem Cells to Uterine Tissue *abs#100*

8:45am **Kam Truong**
Isolation of putative embryonic stem cells from cloned pig embryos *abs#101*

9:00am **Jared Campbell**
The effect of insulin on embryonic stem cell progenitor cells in the mouse blastocyst *abs#102*

9:15am **Tu'uhevaha Kaitu'u-Lino**
Role of candidate stem/progenitor cells in a mouse model of endometrial menstrual breakdown and repair *abs#103*

9:30am **Tiziana Brevini**
Proliferation ability, telomerase activity and molecular characterization of pluripotent cell lines from IVF and parthenogentic pig embryos *abs#104*

9:45am **Sullip Majhi**
Transplanted Germ Cells Can Colonize the Gonads of Sexually Competent Fish and Produce Functional Gametes *abs#105*

SRB Orals: Implantation / Placentation

8:30 AM - 10:00 AM — Meeting Rooms 4 & 5

Chairs: Claire Roberts and Laura Parry

8:30am **Qi Chen**
IL-6 increases the shedding of necrotic trophoblasts from placental explants *abs#106*

8:45am **Eva Dimitriadis**
Interleukin 11 and leukemia inhibitory factor regulate cytokine networks in human first trimester placenta *abs#107*

9:00am **Harmeet Singh**
Functional role of HtrA3 in trophoblast cell invasion during human placental development *abs#108*

9:15am **Kathryn Askelund**
Trophoblast deportation is dependent upon caspases 3, 8 and ROCK *abs#109*

9:30am **YuXia Chen**
Effects of cAMP and serum on human trophoblast cell viability and differentiation *abs#110*

9:45am **Melinda Jasper**
LIF regulation of epithelial cell fucosyltransferase expression in mouse endometrium during early pregnancy *abs#111*

ESA Servier Award Lecture

9:30 AM - 9:45 AM — Hall B

Chair: David Phillips

Presented by: **Kristy Brown**

ESA Morning Tea
9:45 AM - 10:15 AM Hall H

SRB Morning Tea
10:00 AM – 10:30 AM Hall H

ESA Novartis Junior Scientist Award
10:15 AM - 11:45 AM Hall B

Chairs: Mark McLean & Sue Lau

10:15am **Sarina Lim**
Absence of myostatin improves cardiac function following myocardial infarct - animal models *abs#200*

10:30am **Kenneth Ho**
Oral iron chelation increases metabolism and protects against diet-induced obesity *abs#201*

10:45am **Pui Pang**
Investigating non-classical signalling pathways of the androgen receptor *abs#202*

11:00am **Kavitha Ravee Iyer**
SLIRP mediated cross-talk between the androgen receptor and notch signaling pathways in prostate cancer *abs#203*

11:15am **Linda Gallo**
Exercise training early in life prevents pancreatic ß-cell mass deficits in growth restricted male rats *abs#204*

11:30am **Sarah To**
Studies on the epigenetic mechanisms of regulation of the prostanoid receptors EP2 and EP4 in breast cancer *abs#205*

SRB Orals: Gametogenesis and Gamete Function
10:30 AM - 12:00 PM Meeting Rooms 4 & 5

Chairs: Gareth Evans and Melanie Sutton-McDowell

10:30am **Marcos Valdez, Jr.**
Differential Development of Sex-related Characters of the GSP and PNP/DO Chickens after Left-ovariectomy *abs#112*

10:45am **Richard Ivell**
Development of a Method of Seminiferous Tubule Transfection *in vitro* to Define Postmeiotic Gene Regulation *abs#113*

11:00am **Tamer Hussein**
Human cumulus-oocyte complexes secrete cumulus expansion enabling factor(s) *abs#114*

11:15am **Harsha Wechalekar**
Whole body heat exposure induces apoptosis in mouse caudal epididymal spermatozoa *abs#115*

11:30am **Reza Salehi**
Effects of Supplemental Vitamin on the Semen Characteristics of Markhoz Goats during non-breeding season *abs#116*

11:45am **Roozbeh Ardebili**
The comparison of sperm freezability using two egg yolk-free diluents in Zandi ram *abs#117*

SRB Orals: Cell Signalling & Gene Regulation in Female Reproduction
10:30 AM - 12:00 PM Meeting Rooms 10 & 11

Chairs: Rob Gilchrist and Kaye Stenvers

10:30am **Laura Watson**
Characterisation of heparan sulphate proteoglycans in the maturing cumulus oocyte complex *abs#118*

10:45am **Ilona Ciller**
The effect of BMPR-IB immunization in immature female mice *abs#119*

11:00am **Kimberley Tam**
Oxygen regulated gene expression in mouse cumulus cells *abs#120*

11:15am **Christine McIntosh**
Extracellular roles for proregions of mouse BMP15 and GDF9 in vivo *abs#121*

11:30am **Ileana Kuyznierewicz**
Regulation of GDF-9 and GDF-9B by FSH in Preantral Follicle Cultures *abs#122*

11:45am **Davina Cossigny (Rosairo)**
Activin A and Ovarian Follicle Development *abs#123*

SRB Founders Lecture

12:00 PM - 1:00 PM Hall C

Lois Salamonsen
Preparing fertile soil: The importance of endometrial receptivity *abs#005*

ESA Lunch

12:00 PM - 1:00 PM Hall H

ESA Meet the Expert: Disorders of Puberty

12:10 PM - 1:00 PM Meeting Rooms 1 & 2

Chair: Jeffrey Zajac

Christine Rodda
Disorders of Puberty *abs#006*

ESA Orals (Clinical / Basic): Metabolism & Obesity (1)

1:00 PM - 2:30 PM Hall B

Chairs: Margaret Morris & Zane Andrews

1:00pm **Priya Sumithran**
Investigating physiological adaptations to weight loss *abs#206*

1:15pm **Scott Clarke**
Discrete changes in blood flow do not elicit temperature excursions in muscle tissue: further evidence of a thermogenic role for muscle *abs#207*

1:30pm **Paul Lee**
Chronic β—blocker therapy is associated with blunted diet-induced thermogenesis, lower physical activity and obesity *abs#208*

1:45pm **Rhianna Laker**
Late, but not early, exercise restores markers of mitochondrial biogenesis in skeletal muscle of growth restricted rats *abs#209*

2:00pm **Helena Teede**
Polycystic ovarian syndrome in Australian women: Results of the Australian Longitudinal Women's Health study *abs#210*

2:15pm **Melanie Tran**
Being born small programs impaired glucose tolerance in pregnancy *abs#211*

ESA Clinical Case Reports

1:00 PM - 2:30 PM Meeting Rooms 4 & 5

Chairs: Mathis Grossmann & Emma Hamilton

1:00pm **Ie-Wen Sim**
Tumour-induced osteomalacia: Uncovering the culprit *abs#212*

1:15pm **Sybil McAuley**
Delayed Puberty and Diabetes Insipidus in an Adolescent Male *abs#213*

1:30pm **Michael Mond**
Polyuria in Pregnancy *abs#214*

1:45pm **Helen Barrett**
Atypical Pituitary Tumour *abs#215*

2:00pm **Chris Yates**
V Excess and K Depletion *abs#216*

2:15pm **Tang Wong**
A case of ACTH-independent Cushing's syndrome with bilateral adrenal adenomas *abs#217*

ESA Orals (Clinical / Basic): HPA, Neuroendocrine & Bone

1:00 PM - 2:30 PM Meeting Rooms 1 & 2

Chairs: Morton Burt & Jane Ellis

1:00pm **Rachel Davey**
The biological role of the calcitonin receptor to protect against induced hypercalcaemia is mediated predominantly via its actions on osteoclasts, the bone resorbing cells *abs#218*

1:15pm **Thuy Vu**
Effects of Parathyroid Hormone Deficiency and Excess on Cortical and Trabecular Micro-architecture *abs#219*

1:30pm **Vita Birzniece**
Neuroendocrine regulation of growth hormone and androgen status by Selective Oestrogen Receptor Modulators in healthy men *abs#220*

1:45pm **Vita Birzniece**
Evidence for central regulation by estrogen of GH secretion in women: a study of the effects of estrogen receptor antagonism *abs#223*

2:00pm **Caroline Jung**
A longitudinal study on the physiological changes in total plasma cortisol and 24-h urinary free cortisol levels during pregnancy and post-partum, compared with non-pregnant subjects and subjects on low dose oral contraceptive pill *abs#222*

2:15pm **Michael Stowasser**
The role of unilateral adrenalectomy in bilateral primary aldosteronism - a 22 year single centre experience *abs#221*

SRB Lunch

1:00 PM - 2:00 PM Hall H

ESA / SRB Joint Symposium (Basic): Reproductive Cancers

2:00 PM - 4:00 PM Hall C

Chair: Renea Taylor & Darryl Russell

The conference acknowledges the sponsorship of iNova Pharmaceuticals

2:00pm **Henry Jabbour**
Inflammatory pathways in endometrial cancer *abs#007*

2:30pm **Wayne Tilley**
Androgen Receptor Signalling In Breast and Prostate Cancers *abs#008*

3:00pm **Judith Clements**
Kallikrein-related proteases as novel therapeutic targets in prostate and ovarian cancer *abs#009*

3:30pm **Elizabeth Musgrove**
Estrogens and the cell cycle in breast cancer *abs#010*

SRB Orals: From Oocytes to Embryos

2:00 PM - 4:00 PM Meeting Rooms 10 & 11

Chairs: David Gardner and Kylie Dunning

2:00pm **H. Morgan**
Epigenetic reprogramming in zygotes involves the global cytosine demethylation of both the paternal and maternal genomes *abs#127*

2:15pm **Peck Chin**
Regulation of stress protein genes during pre-implantation embryo development in mice by granulocyte-macrophage colony-stimulating factor (GM-CSF) *abs#125*

2:30pm **Sarah Wakefield**
Implications for fetal and placental development following mitochondrial perturbation in the embryo *abs#126*

2:45pm **Y. Li**
DNA Methylation in the 2-cell mouse embryo is the result of DNMT activity during development from the zygote to the 2-cell stage *abs#124*

3:00pm **Alicia Filby**
A role for Sirtuin 3 in the developing mammalian embryo *abs#128*

3:15pm **Michael Bertoldo**
Reduced oocyte developmental competence during the period of seasonal infertility in pigs *abs#129*

3:30pm **Tod Fullston**
Microarray analysis of foetal mouse brain following induction of mitochondrial dysfunction during pre-implantation embryo development *abs#130*

3:45pm **Firas Albuz**
Induced oocyte in vitro maturation (IVM) substantially improves embryo yield and pregnancy outcomes *abs#131*

ESA Symposium (Clinical): Pituitary Disease

2:30 PM - 4:30 PM Hall B

Chair: Warrick Inder & Vita Birzniece

The conference acknowledges the support of Ipsen

2:30pm **Karen Miller**
Hypopituitarism *abs#011*

3:00pm **Michael Dally**
The evolution of radiotherapy practice in the modern management of pituitary tumours *abs#012*

3:30pm **David Torpy**
Management of Cushing's Disease *abs#013*

4:00pm **Emma Duncan**
Pre-treatment of acromegaly and cushings *abs#014*

ESA / Neuroendocrinology Australasia Joint Symposium (Basic): Snake Oil and Serpentine Receptors - Drug Discovery and G Protein Coupled Receptors

2:30 PM - 4:30 PM Meeting Rooms 4 & 5

Chairs: Brian Oldfield & Greg Anderson

The conference acknowledges the support of Beckman Coulter

2:30pm **Walter Thomas**
A GFP complementation screen in living cells to identify novel modifiers of GPCRs *abs#015*

3:00pm **Patrick Sexton**
Amylin receptors: a complex road to drug discovery *abs#016*

3:30pm **Kevin Pfleger**
GPCR heteromerization and drug discovery *abs#017*

4:00pm **Andrew Allen**
Using GPCRs to functionally dissect complex neuronal circuits *in vivo* *abs#018*

Joint ESA / SRB Poster Session

4:15 PM - 6:15 PM Hall H

See poster listing for abstract positions (pages 36 - 41)

Women in Endocrinology Function

6:00 PM – 7:00 PM Riverbank Room 1

The conference acknowledges the support of Beckman Coulter

SRB Students Annual Meeting

6:00 PM – 7:00 PM Meeting Rooms 10 & 11

ESA / SRB Student-Supervisor Function

7:00 PM - 11:30 PM Adelaide Rowing Club

The conference acknowledges the support of Amgen Australia

Tuesday, 25th August 2009

Amgen Australia Breakfast Workshop: Molecular Signalling Pathways as Novel Therapeutic Targets in Bone Health

7:00 AM - 8:20 AM Meeting Room 10

Chair: Peter Ebeling

Jack Martin
Rank ligand and related mechanisms in bone regulation

Rory Clifton-Bligh
The calcium sensing receptor as a therapeutic target in primary hyperparathyroidism: the role of calcimimetics

ESA Plenary Lecture

8:30 AM - 9:30 AM Hall B

Chair: Bu Yeap

Joseph Verbalis
Hyponatremia: 2009 and Beyond *abs#019*

RF & D Award Lecture

8:30 AM - 9:30 AM Hall D

Fulvio Gandolfi
In vitro maturation of farm animals oocytes: a useful tool for investigating the mechanisms leading to full term development *abs#020*

ESA/SRB Morning Tea

9:30 AM - 10:00 AM Hall H

ESA Symposium (Clinical): Endocrine Consequences of Cancer Therapy

10:00 AM - 12:00 PM Hall C

Chairs: Carolyn Allan & Ann McCormack

The conference acknowledges the support of Novartis Oncology

10:00am **Kati Matthiesson**
Androgen deprivation therapy in prostate cancer *abs#021*

10:30am **Margaret Zacharin**
Adult endocrine consequences of childhood cancer *abs#022*

11:00am **John Forbes**
Aromatase inhibitor use for prevention and treatment of endocrine sensitive breast cancer *abs#023*

11:30am **Mark McLean**
Cranial irradiation and pituitary function *abs#024*

ESA Symposium (Basic): Nuclear Hormone Receptors

10:00 AM - 12:00 AM Hall B

Chairs: Tim Cole & Helen MacLean

The conference acknowledges the support of Amgen Australia

10:00am **John Cidlowski**
The Human Glucocorticoid Receptor ß Isoform in Health and Disease *abs#025*

10:30am **George Muscat**
Elucidating RORalpha function: *insights into metabolic disease* *abs#026*

11:00am **Christine Clarke**
Steroid hormone receptors in cancer *abs#027*

11:30am **Peter Fuller**
Molecular mechanisms of the mineralocorticoid receptor *abs#028*

ESA / SRB Joint Orals (Basic): Female Reproduction

10:00 AM - 12:00 PM Hall D

Chair: Greg Anderson & Melanie Mitchell

10:00am **Lachlan Moldenhauer**
Immune-deviating cytokines determine the maternal T cell response during pregnancy and tolerance or rejection of the conceputs *abs#132 (SRB)*

10:15am **Kaushik Maiti**
Role of the cell surface estrogen receptor, GPR-30 in contractility of human pregnant myometrium *abs#224*

10:30am **Janet Holt**
Spatial regulation of APC^{Cdh1} induced cyclin B1 degradation maintains GV arrest in mouse oocytes *abs#133 (SRB)*

10:45am **Roger Smith**
Estrogen receptor α forms strong inter-relationships with expression of inflammatory, contraction-associated and novel genes in the human myometrium before and during labour *abs#225*

11:00am **Maxime Sasseville**
Epidermal growth factor receptor/MAPK3/1 pathway cross-talk enables growth differentiation factor 9 to signal through Smad2/3 in mouse granulosa cells *abs#134 (SRB)*

11:15am **Jeremy Smith**
Hypothalamic expression of the kisspeptin gene (Kiss1) and the RFamide-related peptide (RFRP) gene during the menstrual cycle of a non-human primate *abs#226*

11:30am **Kylie Dunning**
Fatty acid oxidation is essential for oocyte developmental competence *abs#135 (SRB)*

11:45am **Nicolette Hodyl**
Glucocorticoid receptor chaperone molecules and sex-specific sensitivity to cortisol in the human placenta *abs#227*

SRB Orals: Male Reproductive Tract

10:00 AM - 12:00 PM Meeting Room 2

Chair: Kate Loveland and Moira O'Bryan

10:00am **Mark Hedger**
Specificity studies on the immunosuppressive and cytotoxic activities of lysophosphatidylcholines (LPCs) of gonadal origin *abs#136*

10:15am **Brett Nixon**
Elucidation of the molecular mechanisms that underpin capacitation-associated sperm surface remodelling *abs#137*

10:30am **Yan Ru Gao**
Region- and time-dependent changes in structure and cellular turnover in androgen deprived mouse epididymis *abs#138*

10:45am **H. Mudaliar**
The Regulation of TRP53 in Sperm *abs#139*

11:00am **Keng Yih Chew**
The development of the phallus in a marsupial *abs#140*

11:15am **Kee Heng**
Development of a model system to assess the regulation of Leydig stem cell differentiation *abs#141*

11:30am **Guillaume Morin**
Characterization and function of the bull sperm protein Spam1: Two distinct isoforms with distinct roles *abs#180*

11:45am **Natasha Czarny**
The first evidence of high susceptibility to cold shock by the spermatozoa of a marsupial, the fat tailed dunnart (*Sminthopsis crassicaudata*) *abs#143*

ESA Harrison Lecture

12:00 PM - 1:00 PM Hall B

Chair: Leon Bach

John Cidlowski
The glucocorticoid receptor: One gene, many proteins – new mechanisms for tissue specific anti-inflammatory actions of glucocorticoids in health and disease *abs#029*

SRB Lunch

12:00 PM - 1:00 PM Hall H

SRB Meet the Professor

12:10 PM - 1:00 PM Meeting Room 2

Presented by: **Lois Salamonsen**

ESA Lunch

1:00 PM - 2:00 PM Hall H

ESA Meet the Expert: Primary Hyperaldosteronism

1:10 PM - 2:00 PM Hall C

Chair: Michael Stowasser

John Funder
Primary Aldosteronism: Where to Now? *abs#030*

SRB New Investigator Award

1:00 PM – 2:45 PM Hall D

Chairs: Michael Holland and Chris Grupen

1:00pm **Kara Gunter**
Role of RNA-binding protein, Musashi-1 (Msi-1), in Murine Folliculogenesis and Oocyte development *abs#144*

1:15pm **L. Ganeshan**
TRP53 regulates the formation of a pluripotent inner cell mass in the early embryo *abs#145*

1:30pm **Alison Care**
Macrophages are essential for maintenance of early pregnancy through regulation of corpus luteum function *abs#146*

1:45pm **Hassan Bakos**
Paternal Obesity Impairs Sperm Function and Subsequent Embryo and Pregnancy Outcomes *abs#147*

2:00pm **Peter Nicholls**
Hormonal regulation of miRNA in the testis *abs#148*

2:15pm **Catherine Itman**
Smad3 dosage influences testicular maturation *abs#149*

2:30pm **Matthew Dun**
The capacitation induced formation of a multimeric sperm-zona pellucida receptor complex *abs#150*

NZSE Annual General Meeting

1:30 PM - 2:00 PM Meeting Room 2

ESA Orals: CSL Biotherapies Bryan Hudson Clinical Award & NZSE Student Award

The first four listed speakers are finalists for the Bryan Hudson Clinical Award, the last three listed speakers are finalists for the NZSE Student Award

2:00 PM - 4:00 PM Hall B

Chairs: Peter Ebeling & Ken McNatty

2:00pm **Samantha Hutchison**
The effect of endurance exercise without energy restriction on insulin resistance measured by euglycaemic hyperinsulinaemic clamp and body composition in obese women with and without polycystic ovary syndrome *abs#228*

2:15pm **Cherie Chiang**
The use of ACTH in adrenal venous sampling improves lateralization of aldosterone oversecretion in primary aldosteronism patients *abs#229*

2:30pm **Paul Lee**
Brown adipose tissue in humans: Prevalence, anthropometric and metabolic predictors *abs#230*

2:45pm **Emma Hamilton**
Bone Architectural Decay in Males with Prostate Cancer treated with Androgen Deprivation Therapy *abs#231*

3:00pm **Bryony McNeill**
The fetal cotyledon is a major source of maternal circulating C-type natriuretic peptide in ovine pregnancy *abs#232*

3:15pm **Hayden McEwen**
Role of Suppressor of Cytokine Signalling-3 (SOCS-3) in the Neuroendocrine Regulation of Fertility in Mice Fed a High Fat Diet *abs#233*

3:30pm **Alicia Mulligan**
STAT5 is activated by leptin in the hypothalamus but is not required for regulation of fertility *abs#234*

ESA Orals (Clinical / Basic): Androgens & Nuclear Hormone Receptors

2:00 PM - 4:00 PM Hall C

Chairs: Stephen McPherson & Grant Buchanan

2:00pm **Aleksandra Ochnik**
The Synthetic Progestin Medroxyprogesterone Acetate Increases Human Breast Epithelial Cell Proliferation by Disrupting Androgen Receptor Signalling *abs#235*

2:15pm **Kesha Rana**
Increased adiposity in androgen receptor knockout mice *abs#236*

2:30pm **David Handelsman**
Randomized placebo-controlled clinical trial of transdermal dihydrotestosterone gel does not influence prostate growth rate and reduces spinal but not hip bone density over 24 months *abs#237*

2:45pm **Timothy Cole**
Glucocorticoids stimulate catecholamine inactivation in the liver and kidney by direct rapid induction of the sulfotransferase SULT1D1 *abs#238*

3:00pm **Shane Colley**
SRA and its binding partner SLIRP have opposing effects on Apoptosis *abs#239*

3:15pm **Eleanor Need**
Specificity of androgen receptor activity on chromatin is determined by multiple receptor domains *abs#240*

3:30pm **Elspeth Gold**
Vinclozolin exposure *in utero* induces post-pubertal prostatitis and reduces daily sperm production via a reversible hormone regulated mechanism *abs#241*

3:45pm **Tanya Day**
Identification of mechanisms underlying prostate tumorigenesis driven by aberrant androgen receptor signalling *abs#242*

ESA Symposium (Clinical / Basic): Sleep Disorders & Circadian Rhythms

2:00 PM - 4:00 PM Meeting Room 5

Chairs: Brian Oldfield & Margaret Zacharin

2:00pm **Peter Liu**
Hormones, sleep and sleep disordered breathing *abs#031*

2:30pm **Doug McEvoy**
Obstructive sleep apnea, cardiovascular disease and metabolic syndrome *abs#032*

3:00pm **David Kennaway**
The biology of circadian rhythm disruption, sleep disturbance and metabolism *abs#033*

3:30pm **Shantha Rajaratnam**
Melatonin in the regulation of sleep and its efficacy in the treatment of circadian rhythm sleep disorders *abs#034*

SRB Afternoon Tea

3:00 PM - 3:30 PM Hall H

SRB Symposium: Immune – Reproductive System Interactions in Mother, Father & Baby

3:30 PM - 5:30 PM Hall D

Chairs: Sarah Robertson and Rebecca Robker

3:30pm **Mark Hedger**
The role of the Sertoli cell in regulating spermatogenesis, immune responses and inflammatory disease: multiple functions, common mechanisms? *abs#035*

4:10pm **Vicki Clifton**
Sex specific function of the human placenta: Implications for fetal growth and survival *abs#036*

4:50pm **JoAnne Richards**
Immune-like mechanisms associated with ovulation *abs#037*

SRB Orals: Female Fertility; Genes Hormones & Environment

3:30 PM - 5:30 PM Meeting Room 2

Chair: Mai Sarraj

3:30pm **Linda Wu**
Lipotoxicity mediated endoplasmic reticulum stress, mitochondrial dysfunction and apoptosis contribute impaired oocyte quality in response to obesity *abs#151*

3:45pm **Megan Mitchell**
Involvement of TRP53 in SIRT1 function during embryo development *abs#152*

4:00pm **Alexander Sobinoff**
Xenobiotics; Influence on ovarian follicular development *abs#153*

4:15pm **Sokcheon Pak**
Effect of Korean Red Ginseng Extract in Steroid-induced Polycystic Ovary Murine Model *abs#154*

4:30pm **Emily Alvino**
CD44 Signalling in the Cumulus Oocyte Complex during Ovulation *abs#155*

4:45pm **Intan Zulkafli**
Effects of albendazole on ovarian oestrogen synthesis in the rat *abs#156*

5:00pm **Katja Hummitzsch**
Expression patterns of extracellular matrix in mice ovaries *abs#157*

5:15pm **Hossein Yazdani**
Effect of A59V locus in the leptin gene on length of pregnancy in Iranian Holstein cows *abs#158*

ESA Afternoon Tea

4:00 PM - 4:30 PM Hall H

ESA Mid-Career Award Lecture

4:30 PM - 5:00 PM Hall B

Chair: Mark McLean

Rachel Davey

Male Sex Hormones and the Skeleton: Using Genetically Modified Mouse Models to Identify their Mechanisms of Action *abs#038*

ESA Annual General Meeting

5:00 PM - 6:00 PM Hall B

SRB Annual General Meeting

5:30 PM - 6:30 PM Hall D

ESA SRB Conference Dinner

7:00 PM - 11:00 PM National Wine Centre of Australia

The conference acknowledges the support of GlaxoSmithKline

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. The Wine Centre is located on the corner of Botanic and Hackney Roads, Adelaide.

Wednesday, 26th August 2009

ESA Breakfast Career Workshop for Junior Scientists

7:00 AM - 8:25 AM — Meeting Room 1 & 2

Chair: Helen MacLean

7:00am **Vicki Clifton**
Scientific career development for clinicians and basic scientists: A balance act between work, research, family and friends *abs#039*

7:20am **Tom Kay**
Keys to delivering a polished presentation *abs#040*

7:40am **Kate Loveland**
Managing a research lab: Making your career fun and productive *abs#041*

8:00am Q & A

ESA Breakfast Career Workshop for Junior Clinicians

7:00 AM - 8:25 AM — Hall D

Chair: Carolyn Allan

7:00am **Shaun McGrath**
What to do in your third year of Endocrine training *abs#042*

7:20am **Rory Clifton-Bligh**
Should you do a research higher degree? *abs#043*

7:40am **Harvey Newnham**
Issues involved in private practice *abs#044*

8:00am Q & A

ESA / ADS Plenary Lecture

8:30 AM - 9:30 AM — Halls B & C

Chairs: Jenny Gunton & Helen MacLean

Mark Febbraio
Role of Myokines in Metabolic Regulation *abs#045*

SRB Orals: Developmental Origins of Health and Disease

8:30 AM - 10:30 AM — Meeting Rooms 10 & 11

Chairs: Brendan Waddell and Karen Kind

8:30am **Patricia Grant**
Maternal nutrition and gestational age affect placental microRNA expression in the guinea pig *abs#159*

8:45am **Wee-Ching Kong**
The potential role of microRNAs in the development of the human placenta in early pregnancy *abs#160*

9:00am **Prabha Andraweera**
Paternal and fetal single nucleotide polymorphisms in KDR gene associate with preeclampsia and intrauterine growth restriction *abs#161*

9:15am **Brooke Pearce**
The effect of maternal folic acid supplementation throughout pregnancy on neurodevelopment, motor function and behaviour of progeny in the rat *abs#162*

9:30am **Wing Hong Chu**
Maternal Folic Acid Supplementation Induced Alterations in Metabolic Health of Progeny: Role of MicroRNA Regulatory Networks *abs#163*

9:45am **Jessica Stringer**
The imprint status and expression of *INS* in the tammar wallaby, *Macropus eugenii* *abs#164*

10:00am **Ying Liu**
Plasticity in imprinting of bovine *IGF2R* correlates with total expression levels in fetal tissues *abs#165*

10:15am **Tina Bianco-Miotto**
Maternal obesity is associated with an increased incidence of prostate abnormalities in adult rat offspring *abs#166*

SRB Symposium: Sperm and Male Reproductive Function

8:30 AM - 10:30 AM Meeting Room 1 & 2

Chairs: Eileen McLaughlin and Julia Young

8:30am **Steve Johnston**
Future proofing Australia's mammalian biodiversity using genome resource banking and ART: Where are we up to? *abs#046*

9:10am **John Fitzpatrick**
Sperm competition, inbreeding and the evolution of superior ejaculates *abs#047*

9:50am **Gareth Evans**
Functional differences between sex-sorted and non-sorted sperm *abs#048*

ESA Symposium (Basic): Bioinformatic Approaches to Research

9:30 AM - 11:00 AM Meeting Rooms 4 & 5

Chairs: Peter Stanton & Rick Nicholson

The conference acknowledges the support of Sanofi-Aventis

9:30am **Nikolai Petrovsky**
Integrating informatic and molecular approaches *abs#049*

10:00am **Terry Speed**
Making the most of gene array data: lessons from the ER *abs#050*

10:30am **Christopher Ormandy**
Application of transcript profiling to the discovery of the genetic regulatory networks mediating development and carcinogenesis of the mammary gland *abs#051*

ESA Symposium (Clinical): Management of the Infertile Couple

9:30 AM - 11:00 AM Hall D

Chair: Louise Hull and Samantha Hutchison

9:30am **Robert Norman**
Polycystic ovary syndrome *abs#052*

10:00am **David Healy**
IVF and the Infertile Female *abs#053*

10:30am **Robert McLachlan**
Management of male infertility *abs#054*

ESA / ADS Symposium (Basic): Endocrine Regulation of Metabolic Cross-Talk

9:30 AM - 11:00 AM Halls B & C

Chairs: Jon Whitehead & Brian Oldfield

9:30am **Zane Andrews**
New insights into the regulation of food intake and adiposity by ghrelin *abs#055*

9:50am **Bronwyn Hegarty**
Adiponectin and AMP-activated protein kinase - challenging the dogma *abs#056*

10:10am **Vance Matthews**
Brain derived neurotrophic factor as a potential anti-obesogenic drug target *abs#057*

10:30am **Matthew Watt**
Pigment-epithelium derived factor contributes to metabolic dysfunction and insulin resistance in obesity *abs#058*

SRB Morning Tea
10:30 AM - 11:00 AM Hall H

ESA / SRB Joint Orals (Basic): Male Reproduction
11:00 AM - 1:00 PM Meeting Rooms 10 & 11

Chairs: Jeremy Smith & Mark Hedger

11:00am **Louisa Ludbrook**
Mutant Steroidogenic Factor-1 from patients with Disorders of Sex Development show reduced activation of the testis-specific enhancer of SOX9 *abs#167 (SRB)*

11:15am **Charles Allan**
Estradiol induction of spermatogenesis is mediated via an ERα and not ERß mechanism involving neuroendocrine activation of FSH secretion *abs#243*

11:30am **Kate Redgrave**
Identification and characterisation of surface protein complexes in human spermatozoa *abs#168 (SRB)*

11:45am **Preetika Balanathan**
Elevated level of inhibin-α subunit is pro-tumourigenic and pro-metastatic and associated with extracapsular spread in advanced prostate cancer *abs#244*

12:00pm **Charles Allan**
Sertoli cell-specific disruption of the androgen receptor DNA-binding domain reveals differential temporal control of distinct androgen-regulated genes *abs#169 (SRB)*

12:15pm **Yao Wang**
Autocrine inhibins regulate immature mouse Leydig cell behaviour *abs#245*

12:30pm **Julia Young**
TGFß signaling in an in vitro seminoma model *abs#170 (SRB)*

ESA Morning Tea
11:00 AM - 11:30 AM Hall H

ESA Orals (Clinical / Basic): Metabolism & Obesity (2)
11:30 AM - 1:30 PM Riverbank 2

Chairs: Paul Lee & Belinda Henry

11:30am **Bich Tran**
Association between common variants in the FTO gene, type 2 diabetes and mortality *abs#246*

11:45am **Himawan Harryanto**
Role of microRNAs in placental programming of insulin resistance *abs#247*

12:00pm **Jacques Beltrand**
Resistance to the effects of leptin replacement therapy of immunological origin in children with Berardinelli Seip congenital lipodystrophy (BSCL) *abs#248*

12:15pm **Belinda Henry**
High cortisol responsiveness may be a marker for the innate predisposition to become obese *abs#249*

12:30pm **Lawrence Blonde**
Monotherapy with Liraglutide, a Once-Daily Human GLP-1 Analogue, Provides Sustained Reductions in HbA_{1C}, FPG, and Weight Compared with Glimepiride in Type 2 Diabetes: LEAD-3 mono 2-year Results *abs#250*

12:45pm **Megan Evetts**
Tetrahydrocannabinol (THC) attenuates weight loss in an activity based model of Anorexia Nervosa *abs#251*

1:00pm **Heshan Peiris**
The role of an endogenous inhibitor of calcineurin in the regulation of glucose homeostasis *abs#252*

1:15pm **Saidatul Mohammad**
Maternal folic acid supplementation in the rat improves insulin disposition in adult male offspring but impairs that of female offspring *abs#253*

ESA Orals (Basic): Development & Carcinogenesis

11:30 AM - 1:30 PM Riverbank 1

Chairs: Vince Russo & Lisa Butler

11:30am **Leon Bach**
MAP kinase pathways are involved in IGF-independent, IGFBP-6-induced rhabdomyosarcoma cell migration *abs#254*

11:45am **Sarah Wilkinson**
Hedgehog signalling implicated in stromal-mediated lineage commitment of adult epithelial cells *abs#255*

12:00pm **Grant Buchanan**
Chip-sequencing of steroid receptors in breast cancer *abs#256*

12:15pm **Rhianna Laker**
Effects of restriction of prenatal and postnatal growth on skeletal muscle development *abs#257*

12:30pm **Kristy Brown**
The regulation of aromatase in the breast by the LKB1/AMPK pathway provides a link between obesity and breast cancer in postmenopausal women *abs#258*

12:45pm **Margaret Centenera**
Combining a Hsp90 Inhibitor with Other Agents that Abrogate Androgen Signalling Synergistically Induces Death of Prostate Cancer Cells *abs#259*

1:00pm **Mary Wlodek**
Being born small reduces the number of cardiomyocyte nuclei which can be increased by improving postnatal nutrition and growth *abs#260*

1:15pm **Maree Bilandzic**
Betaglycan regulates TGF-beta Superfamily Action in Human Granulosa Tumour Cells *abs#261*

ADS / SRB Symposium: Diet and Inherited Characteristics

11:30 AM - 1:00 PM Hall D

Chairs: Larry Chamley and Sue Mei Lau

The conference acknowledges the support of Eli Lilly

11:30am **Kathryn Gatford**
Poor growth before birth impairs insulin secretion – what we have learnt about the mechanisms from the placentally-restricted sheep *abs#059*

12:00pm **Margaret Morris**
Over feeding early in life and risk of obesity: Insight from the rodent *abs#060*

12:30pm **Claire Roberts**
Paternal factors influencing gestational outcome in offspring *abs#061*

SRB Lunch

1:00 PM - 2:00 PM Hall H

ESA Lunch

1:30 PM - 3:00 PM Hall H

ESA Meet the Expert: Male Contraception

2:00 PM - 3:00 PM — Meeting Rooms 10 & 11

Chair: Mathis Grossmann

Presented by: **David Handelsman**

SRB / ADS Plenary

2:00 PM - 3:00 PM — Halls B & C

Chair: Julie Owens

Rebecca Simmons

Developmental origins of type 2 diabetes: epigenetic mechanisms in beta cell failure *abs#062*

NZSE/ESA Nancy Sirett Lecture

3:00 PM - 4:00 PM — Hall D

Chair: Graham Barrell

Peter Gluckman

Maternal, trans-generational and epigenetic contributions to metabolic disease *abs#063*

SRB Orals: Uterine Function

3:00 PM - 4:30 PM — Riverbank 2

Chairs: Tu Uhevaha Lino and Jane Girling

3:00pm **Sarah Paule**
Identification of decidualisation- induced protein changes in human endometrial stromal cells by proteomics *abs#171*

3:15pm **Ellen Menkhorst**
Development of a non-hormonal contraceptive: Vaginal delivery of a LIF inhibitor: its tissue distribution and effect on implantation in mice *abs#172*

3:30pm **Jemma Evans**
Endometrial repair - enter the matrix *abs#173*

3:45pm **Jane Fenelon**
The epidermal growth factor (EGF) family in the endometrium and blastocyst of the tammar wallaby, *Macropus eugenii* during embryonic diapause *abs#174*

4:00pm **Sophea Heng**
The role of proprotein convertase 6 during decidualization: regulation of bone morphogenetic protein 2 activation *abs#175*

4:15pm **David Sharkey**
GDF-9, BMP-15 and Activin A contribute to seminal fluid signalling in human cervical epithelial cells *abs#176*

SRB Orals: Cell signalling & Gene Regulation in Male Reproduction

3:00 PM – 4:15 PM — Riverbank 1

3:00pm **Louisa Ludbrook**
A mechanism underlying Disorders of Sex Development caused by *DAX1* duplication *abs#177*

3:15pm **Wendy Winnall**
Rat testicular macrophages exhibit an "alternatively activated" response to lipopolysaccharide (LPS), interferon-γ (IFNγ) and interleukin-4 (IL-4), consistent with immune privilege *abs#178*

3:30pm **Elspeth Gold**
Characterisation of the *in vitro* function of activin AC *abs#179*

3:45pm **Melissa Gamat**
Hepatocyte-nuclear factor 3-alpha (HNF-3α) expression in the developing prostate of the tammar wallaby: a marker of prostate differentiation *abs#181*

4:00pm **Michael Morris**
Novel signalling in mouse embryonic stem cells generates primitive ectoderm-like cells *abs#182*

ESA Japan-Australia Plenary Lecture

4:00 PM - 5:00 PM Hall D

Chair: Evan Simpson

Hironobu Sasano
Intratumoral estrogen production in human breast cancer - its regulation and inhibition - *abs#064*

For the treatment of acromegaly and symptoms of carcinoid syndrome

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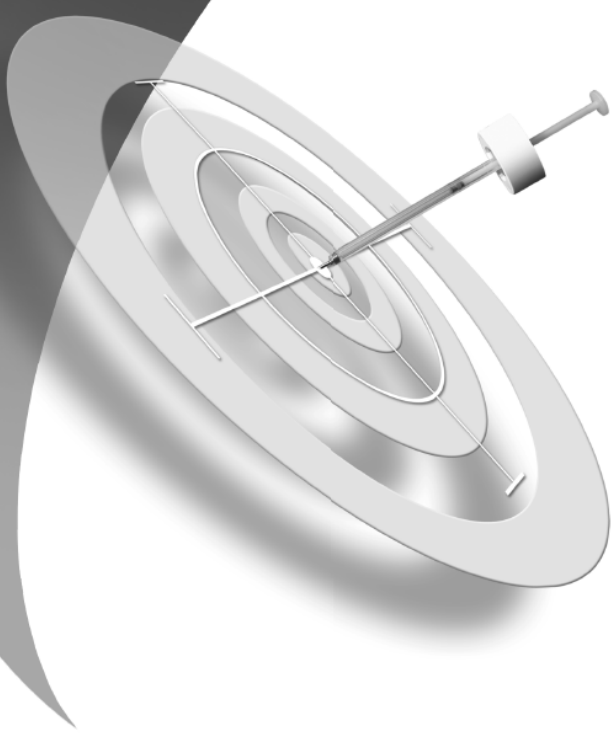

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Before prescribing please refer to full Product Information, which is available from Ipsen Pty Ltd.

Somatuline® Autogel®: lanreotide as acetate in a pre-filled syringe (60, 90 & 120 mg). **Indications**: the treatment of acromegaly when circulating growth hormone and IGF-1 levels remain abnormal after surgery and/or radiotherapy or in patients who have failed dopamine agonist therapy; the treatment of symptoms of carcinoid syndrome associated with carcinoid tumours. **Contraindications**: lactation; hypersensitivity to lanreotide or related peptides. **Precautions**: may experience hypoglycaemia or hyperglycaemia (monitor blood glucose levels); may reduce gall bladder motility (recommend gall bladder echography); exclude presence of obstructive intestinal tumour; monitor kidney and liver function; may reduce heart rate in patients with an underlying cardiac problem (monitor heart rate). Not recommended for use in children. See full PI for further information. **Adverse Events**: common to very common: fatigue, headache, dizziness, sinus bradycardia, hypoglycaemia or hyperglycaemia, anorexia, diarrhoea, abdominal pain, nausea, vomiting, dyspepsia, flatulence, cholelithiasis, bilirubin increase, injection site reaction. See full PI for further information. **Dose**: Acromegaly: for first time treatment the starting dose is 60 mg every 28 days; for patients previously treated with Somatuline LA every 14, 10 or 7 days, the starting dose is 60 mg, 90 mg or 120 mg respectively every 28 days. Dosage should be adjusted according to GH and/or IGF-1 response. Patients well controlled on lanreotide can be treated with 120 mg every 42–56 days. Carcinoid syndrome: 60 to 120 mg every 28 days, adjusted according to symptomatic relief. **Administration**: deep subcutaneous injection in the superior external quadrant of the buttock (healthcare professional or carer); or the upper, outer thigh (self-administration). **Storage**: 2°C–8°C. **Date of TGA approval**: 30 July 2007.

*Effective biochemical control defined as normalised IGF-1 and GH $\leq$ 2.5 µg/L.[2–5]
†6-month, open, uncontrolled dose-titration study.[6]

References
1. Somatuline Autogel Product Information, 30 July 2007.
2. Caron P *et al*. *J Endocrinol Metab* 2002;**87**(1):99–104.
3. Caron P *et al*. *Clin Endocrinol* 2004;**60**(6):734–40.
4. Caron P *et al*. *Clin Endocrinol* 2006;**64**(2):209–14.
5. Chanson P *et al*. *Clin Endocrinol* 2008;**69**(2):299–305.
6. Ruszniewski P *et al*. *Neuroendocrinology* 2004;**80**(4):244–51.

For further information about Somatuline Autogel, contact Ipsen Pty Ltd: T (03) 8544 8100 F (03) 9562 5152 E info@ipsen.com.au
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ESA POSTER LISTING

Monday August 24th, 4:15 PM – 6:15 PM

Adrenal

Morton Burt

Comparison of fasting glucose with the oral glucose tolerance test to screen for diabetes in subjects receiving chronic low dose glucocorticoid therapy *abs#300*

Daniel Chen

An audit of genetic screening practices for phaeochromocytoma and paraganglioma *abs#301*

Lucia Gagliardi

Screening for adrenocortical hypersecretory states in type 2 diabetes mellitus: low false-positive rates with nocturnal salivary cortisol and aldosterone-renin ratio *abs#302*

Jui Ho

Plasma Nitrate/nitrite (NOx) correlated to Sepsis Severity and Mortality but not Vasopressor Requirement *abs#303*

Eduardo Pimenta

Should treatment for glycocorticoid-suppressible hyperaldosteronism (GSH) be commenced long before hypertension develops, and, if so, which? *abs#304*

Matthew Rutherford

The -344C/T polymorphism in the aldosterone synthase gene (*CYP11B2*) and left ventricular remodelling following myocardial infarction *abs#305*

Sandie Staermose

Elevated serum interleukin 6 levels in normotensive individuals with Familial Hyperaldosteronism Type 1 *abs#306*

Paul Taylor

A high-throughput mass spectrometric method for aldosterone measurement: A step towards improvement diagnosis of primary aldosteronism *abs#307*

Androgens

Miriam Butler

Expression of Small Glutamine-Rich Tetratricopeptide Repeat-Containing Protein Alpha (SGTA) in Relation to Androgen Receptor Expression and Subcellular Localisation in Human Ovarian Tissues and Ovarian Cancer Cell Lines *abs#308*

D Harwood

Development and validation of a sensitive liquid chromatography-tandem mass spectrometry assay to simultaneously quantify androgens and estrogens from serum *abs#309*

Scott Johnston

Impact of neonatal testosterone exposure on the physiology and stress induced cortisol secretion of gonadectomized piglets *abs#310*

Andrew Trotta

SGTA as a regulator of androgen receptor signalling in prostate cancer *abs#311*

Bone

Frances Milat

The effects of parathyroid hormone and parathyroid hormone-related protein on WNT signalling and other signalling pathways in osteoblasts *abs#312*

Anuradha Sakthivel

Bone density and Body Composition in Spina Bifida *abs#313*

Christy Sankoorikal

Atypical Femoral Shaft fractures in a post menopausal women after long term bisphosphonate therapy *abs#314*

Vasant Shenoy

Bone mineral density in Indigenous Australians: eGFR study pilot results *abs#315*

Sunethra Thomas
Vitamin D status in the third trimester and newborns in a South Australian cohort *abs#316*
Robert Tuckey
Metabolism of substrates incorporated into phospholipid vesicles by 25-hydroxyvitamin D3 1α-hydroxylase (CYP27B1) *abs#317*

Cancer

Kara Britt
The role of the mammary stroma in parity-induced breast cancer protection *abs#318*
Lisa Butler
Synergistic induction of apoptosis in prostate cancer cells by combinations of agents that suppress androgen receptor signalling *abs#319*
Karen Chiam
Changes in DNA methylation and expression status of GSTP1 is a marker of treatment response to epigenetic therapy in prostate cancer *abs#320*
Qihan Dong
Role of Cytosolic PhospholipaseA2-α in Prostate Cancer Cell Proliferation *abs#321*
Stacey Jamieson
Constitutive NF-κB signalling may play a role in the molecular pathogenesis of granulosa cell tumours of the ovary *abs#322*
Tanja Jankovic-Karasoulos
Testosterone supplementation during anastrozole therapy does not increase proliferation in postmenopausal human breast tumour tissue *abs#323*
Shantha Joseph
Peri-operative Assessment of the Hypothalamic / Pituitary / Adrenal (HPA) Axis in Patients with Pituitary Adenomas- an Australasian Survey *abs#324*
Kevin Knower
Epigenetic regulation of local estrogen biosynthesis in human breast adipose fibroblast cells *abs#325*
Roxanne Toivanen
A new method for xenografting primary human prostate cancer using tissue recombination *abs#326*

Clinical

Dan Harmelin
Medical management of primary hyperparathyroidism: Is PBS funding necessary? *abs#327*
Flora Ip
A case of lung adenocarcinoma metastatic to the pituitary gland causing central diabetes insipidus *abs#328*
Narsing Laddipeerla
Hypophosphatemic Osteomalacia: A case report *abs#329*
Sarina Lim
Mother and daughter with pituitary adenomas: Familial or co-incidental? *abs#330*
Usman Malabu
High prevalence of hyponatraemia amongst acute medical admissions in Tropical North Queensland *abs#331*
Gregory Ong
Case report: Complex management of refractory hypoglycaemia in a man with malignant insulinoma *abs#332*
Lisa Simmons
Cushing's Syndrome in an IVF pregnancy *abs#333*

Genetics

Nick Hatzirodos
An investigation of the fibrillin-3 gene in polycystic ovary syndrome and expression of the fibrillin gene family in human ovarian tissue *abs#334*

GH/IGF

Bu Yeap

Insulin-like growth factor 1 and its binding proteins 1 and 3 are differentially associated with metabolic syndrome in older men. The Health In Men Study. *abs#335*

Jingting Zhao

Identification of growth hormone-regulated genes in adipose tissue: a microarray analysis in hypopituitary men *abs#336*

HPA

Sinan Ali

In-Vivo Cleavage of human corticosteroid binding globulin (CBG) in sepsis patients *abs#337*

Sinan Ali

Phosphorylation contributes to human corticosteroid binding globulin (CBG) heterogeneity *abs#338*

Julie Hetherington

Performance of Glucose Meters during Hypoglycaemic Dynamic Testing *abs#339*

Warwick Howe

Baseline serum cortisol in predicting adrenal sufficiency as assessed by the 250 mcg Synacthen Stimulation Tests (SST) in 625 patients *abs#340*

Peter Mark

Glucocorticoid receptor null mouse placentas are larger and show reduced mean harmonic thickness relative to wildype littermates *abs#341*

Linda Mignone

Management Survey of Addison's Disease during pregnancy: Relation to self-reported pregnancy outcomes *abs#342*

Metabolism & Obesity

Lawrence Blonde

Switching from Twice-Daily Exenatide to Once-Daily Liraglutide Improves Glycemic Control in T2D on Oral Agents *abs#343*

Jenny Chow

The generation of a doxycycline-inducible, tissue-specific aromatase transgenic mouse *abs#344*

Balasubramanian Krishna Murthy

Pre-proinsulin Specific T cells can be Isolated from the Islets of a T1D Pancreas *abs#345*

Margaret Morris

Effects of different levels of maternal overnutrition on offspring early in life *abs#346*

Sachithanandan Nirupa

Use of Novel Biochemical and Traditional Clinical Risk Factors to Estimate Insulin Resistance in Type 1 Diabetes *abs#347*

Sarah Spencer

Early life overfeeding leads to obesity in adulthood and altered neuroimmune responses to lipopolysaccharide *abs#348*

Ya Tuo

Mitochondrial mediated apoptosis by chronic exposure of INS-1 rat insulin cells to high levels of linoleic acid and glucose *abs#349*

Michelle Van Sinderen

Role of Sex Hormones in Metabolic Syndrome - lessons from the Aromatase Knockout (ArKO) mouse *abs#350*

Alexander Viardot

Prader-Willi Syndrome is associated with activation of the innate immune system independently from central adiposity and insulin resistance *abs#351*

Neuroendocrinology

Jessica Jacobi

Evidence for a rapid effect of estradiol on Kisspeptin Cells in the Ewe *abs#352*

Zhi Yi Ong

Is junk food addictive? The role of the mesolimbic reward pathway *abs#353*

Ika Puspita Sari

Reduced RF-amide related peptide (RFRP) gene expression in the follicular phase of the ewe estrous cycle permits increased LH secretion from gonadotropes *abs#354*

Simon Rajaratnam

Our experience of prolactinomas in males *abs#355*

Sunita Sandhu

Phaeochromocytoma, neurofibromatosis & GIST: A rare combination? *abs#356*

Lei Zhang

Peripheral Neuropeptide Y Y1-Receptors Regulate Lipid Oxidation and Fat Accretion *abs#357*

Nuclear Hormone Receptors

Christine Clarke

Disruption of nuclear structure alters progesterone signalling in breast cells *abs#358*

Dennis Dowhan

Protein Arginine Methyltransferase 6 (PRMT6) is Involved in Steroid Hormone Receptor Mediated Gene Expression in Breast Cancer Cells *abs#359*

Isabelle Hoong

Role of glucocorticoids in the regulation of adipogenesis *abs#360*

Carolyn Mitchell

Molecular mechanisms controlling Prostaglandin Endoperoxide Synthase-2 (PTGS2) gene activity by glucocorticoid in term amnion *abs#361*

Pregnancy & Parturition

Michelle Colomiere

Human placental lactogen regulates pregnancy induced insulin resistance in human subcutaneous adipose tissue *abs#362*

Sarah Holdsworth-Carson

Pro-inflammatory cytokine release and matrix metalloproteinase activity in response to PPAR gamma-activation in LPS-stimulated human gestational tissues *abs#363*

Damien Keating

Role of the Vesicular Chloride Transporter ClC-3 in Secretion from Endocrine Cells *abs#364*

Jessica Lewis

Ligand-induced activation of PPARα and PPARγ upregulates expression of antioxidant defences in placental BeWo cells *abs#365*

Eugenie Lumbers

Levels of expression of components of the renin-angiotensin system (RAS) in fetal membranes, decidua and placenta and the effects of gender and labour *abs#366*

Annette Osei-Kumah

Placental micro RNA expression in pregnancies complicated by asthma *abs#367*

Jonathan Paul

Development of myometrial contractility in humans is associated with phosphorylation of cofilin and caldesmon *abs#368*

Sumathy Perampalam

Prevalence of Vitamin D deficiency in pregnancy in 2 Australian populations *abs#369*

John Schjenken

A new mechanism for CRH-induced immunosuppression during pregnancy *abs#370*

Michael Stark

The influence of antenatal glucocorticoids and fetal sex on preterm placental oxidant and anti-oxidant status *abs#371*

Penny Wolski

The use of Lugol's iodine for the control of severe thyrotoxicosis in pregnancy *abs#372*

Receptor/Signal Transduction

Ilona Ciller

BMPR-IB neutralization in male mice reveals a regulatory role for the type I receptor in gonadotrophin stimulated androgen secretion *abs#373*

Soohyun Lee

Identification of G-protein coupled receptors (GPCRs), GPR40 and GPR120 in human endometrial cancer cell lines and their potential regulation of cell proliferation *abs#374*

Jun Yang

The mineralocorticoid receptor: Exploring ligand-specificity using peptide phage display *abs#375*

Female Reproduction

Vicki Edwards

Assessment of the reproductive toxicity of compounds from the marine mollusc *Dicathais orbita* *abs#376*

Seng Liew

Estrogen dependent gene expression in the mouse ovary *abs#377*

Lisa Moran

Polycystic Ovary Syndrome: A biopsychosocial understanding in young adult women to improve knowledge and treatment options *abs#378*

Melissa Papargiris

Gonadotrophin inhibitory hormone (GnIH) neurones are not activated by psychosocial stress in the ewe *abs#379*

Roger Smith

Apoptosis in pregnant myometrium and its role in labour *abs#380*

Astrud Tuck

Kit Ligand Expression and Regulation in Human Ovarian Granulosa Cells *abs#381*

Dina Zebian

Effects of the cyanotoxin CYN, on human granulosa cell viability, hormone production and protein synthesis *abs#382*

Male Reproduction

Caroline Foo

A global analysis of androgen-regulated proteins in rat testis *abs#383*

Gerard Gibbs

Cysteine-Rich Secretory Protein (CRISP)-4 inhibition of the Transient Receptor Potential Melastatin member 8 (TRPM8) ion channel: Involvement in sperm function and implications for male fertility *abs#384*

Peter Liu

Testosterone's short-term positive effect on luteinising hormone secretory burst mass and its negative effect on secretory burst frequency are attenuated in middle-aged men *abs#385*

Ulla Simanainen

Glucocorticoid receptor expression and effect of corticosterone on mouse prostate *abs#386*

Reproductive Hormones

Ravinder Anand-Ivell

Postnatal development and dynamic of the secreted Leydig cell peptide hormone Insulin-like peptide 3 (INSL3) in rodents *abs#387*

Allan Bond

Effect of cryopreservation on ovine luteal cells steroidogenesis *abs#388*

Ursula Ciller

Variation in adult murine Leydig cell testosterone production by different isoforms of equine chorionic gonadotropin using interstitial cell culture *abs#389*

Lisa Martin

Steroid production controlled by protein assembly on membranes *abs#390*

Keely McNamara

Quantification of multiple steroid hormones in male mouse serum and reproductive tissue using an LC-MS/MS assay *abs#391*

Courtney Simpson

Purification and Function of Recombinant Murine Growth Differentiation Factor-9 *abs#392*

Kelly Walton

Molecular interactions that govern the synthesis, secretion and extracellular matrix deposition of TGF-β1 *abs#393*

Hui Wu

What is the source of activin A release during acute inflammation? Assessment of tissue mRNA and protein levels following lipopolysaccharide challenge *abs#394*

Tamas Zakar

Prostaglandin transporter (hPGT) and 15-hydroxyprostaglandin dehydrogenase (PGDH) gene expression decreases in a coordinate fashion in the human decidua with labour *abs#395*

Pawel Zarzycki

Multivariate classification and characterization of water ecosystems based on high throughput analysis of endocrine disrupting compounds using temperature-dependent inclusion chromatography *abs#396*

Thyroid

Suzanne Brown

Thyroid Function Tests Do Not Improve the Predictive Value of First Trimester Screening for Preterm Delivery *abs#397*

Ada Cheung

Reversible pancytopenia with treatment of thyrotoxicosis due to Graves' disease *abs#398*

M Ellis

Access and immulite thyroglobulin assay differences in the presence of anti-thyroglobulin antibodies *abs#399*

Stephen Fitzgerald

Psychological distress decreases from high to normal levels over twelve months in graves' disease *abs#400*

Stephen Fitzgerald

Preceding stressful life events and subsequent remission in graves' disease *abs#401*

Kiernan Hughes

Accuracy of pre-operative localisation of parathyroid adenoma using 99mTc-pertechnetate sestamibi scan and ultrasound in patients with primary hyperparathyroidism undergoing parathyroidectomy *abs#402*

Eun-Sook Kim

The association of anti-thyroglobulin antibody with increased cancer risk in thyroid nodules *abs#403*

John Kok

Compliance with thyroid function monitoring in patients on amiodarone *abs#404*

Agata Piotrowicz

A four year audit of thyroid cytology from a single endocrinologists' practice *abs#405*

Jimmy Shen

Thyroid cancer in Graves' Disease: An update on evaluation and management *abs#406*

John Walsh

Risk factors for hypothyroidism: a 13 year, longitudinal analysis of a community-based cohort using current assay methodology *abs#407*

Powerful Partners, in glycaemic control.[1-4]

LANTUS® insulin glargine SoloSTAR®

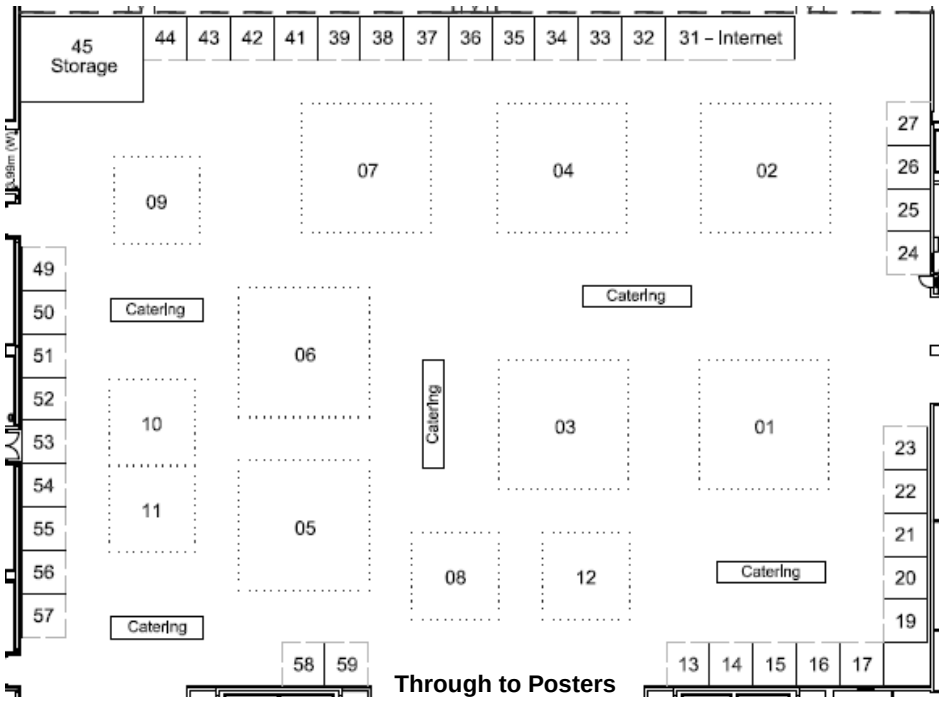
APIDRA® insulin glulisine SoloSTAR®

Lantus® (insulin glargine). Indications: Once-daily subcutaneous administration for type 1 diabetes mellitus patients (adults and children) and type 2 diabetes mellitus patients (adults) who require insulin for control of hyperglycaemia. Contraindications: Hypersensitivity to insulin glargine or any excipient. Precautions: Hypoglycaemia, hepatic, renal and visual impairment; lipodystrophy and other injection site; antibody production; intercurrent conditions, not studied in children < 6 years, pregnancy category B3, lactation; not intended for i.v. use; not recommended for treatment of diabetic ketoacidosis; LANTUS® MUST NOT BE DILUTED OR MIXED WITH ANY OTHER INSULIN OR SOLUTION. Interactions: Oral antidiabetic agents; cardiovascular, analgaesic, anti-inflammatory, neurological, antipsychotic agents (see full PI); antibiotics; corticosteroids, other hormonal therapies (see full PI); diuretics; protease inhibitors; sympathomimetic agents; lithium; alcohol; sympatholytics including beta-blockers; others, see full PI. Side effects: Hypoglycaemia; injection site reactions; visual disturbances; others, see full PI. Dosage and Administration: Subcutaneous, once daily. Lantus is equipotent to human insulin. Initial dose should be determined depending on desired blood glucose levels and doses and timing of any antidiabetic medication. For changeover from once-daily NPH or ultralente, initial dose usually not changed; for changeover from twice-daily NPH to once-daily Lantus, initial dose usually reduced by approximately 20% compared to total daily NPH dose; for initiation of type 2 patients, initial dose is usually approximately 10IU. Please review full Product Information before prescribing Lantus®. Full Product Information is available on request from sanofi-aventis australia pty ltd, 12-24 Talavera Road, Macquarie Park NSW 2113. ABN 31 008 558 807. Date created: 30 May 2007. Reference Document: PI, approved 25 May 2006. Lantus® and Lantus® SoloSTAR® are a registered trademark of sanofi-aventis. Apidra® Minimum Product Information. Apidra® (insulin glulisine). Indications: For type 1 and type 2 diabetes mellitus patients (adults and children older than 12 years) who require insulin for control of hyperglycaemia. Contraindications: Hypersensitivity to insulin glulisine or any excipient. Precautions: Hypoglycaemia; hepatic, renal and visual impairment; lipodystrophy and other injection site reactions; antibody production; intercurrent conditions; not studied in children < 12 years, pregnancy category B3, lactation. Apidra® may be mixed with NPH insulin; mixtures should not be administered intravenously. Interactions: Oral antidiabetic agents; cardiovascular, analgaesic, anti-inflammatory, neurological, antipsychotic agents (see full PI); antibiotics; corticosteroids, other hormonal therapies (see full PI); diuretics; protease inhibitors; sympathomimetic agents; lithium; alcohol; sympatholytics including b-blockers; others, see full PI. Side effects: Hypoglycaemia; injection site reactions; others, see full PI. Dosage and Administration: Apidra® is equipotent to human insulin, with more rapid onset and shorter duration of action. Apidra® should be injected within 15 minutes before or immediately after a meal. Please review Product Information before prescribing. Full Product Information is available on request from sanofi-aventis australia pty ltd, 12-24 Talavera Road, Macquarie Park NSW 2113. ABN 31 008 558 807. Date created: 30 May 2007. Reference Document: PI, approved 15 November 2006. Apidra® SoloSTAR® is a registered trademark of sanofi-aventis. AVEN5341. AU.GLA.09.01.003. References: 1. Lantus Product Information. Approved 21 January 2008. 2. Apidra Production Information. Approved 24 June 2008. 3. Ruhnau KJ et al. IDF 2006. Abstract P952. 4. Lankisch M et al. 2007 ADA Abstract . 0198-OR.

PBS Information: Lantus® SoloSTAR® and Lantus® cartridges are listed on the PBS as a long acting insulin analogue for the treatment of type 1 and type 2 diabetes. Apidra® SoloSTAR® and Apidra® vial are listed on the PBS as a rapid acting insulin analogue for the treatment of type 1 and type 2 diabetes.

sanofi aventis

Because health matters



Abbott Diabetes Care **Booth 7**

666 Doncaster Road
Doncaster VIC 3109
Ph: 03 9843 7135, 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 monitors which help people manage their diabetes and provide flexible lifestyle choices.

Allergan Australia Pty Ltd **Booth 19**

Level 4, 810 Pacific Highway
Gordon NSW 2072
Ph: 02 9498 0224, Fax: 02 9498 0184
Contact: Elizabeth Nathan
Email: nathan_elizabeth@allergan.com
Web: www.allergan.com.au

Allergan Australia Pty Ltd offers two weight management systems designed to help overweight and obese patients lose weight, in conjunction with a health diet and lifestyle.

The LAP-BAND® System is a laparoscopic gastric banding procedure suitable for obese patients. The gastric band is reversible, adjustable and minimally invasive. The LAP-BAND® System has been clinically proven to resolve type II diabetes and other obesity related diseases.

The ORBERA® Managed Weight Loss System is a temporary, non surgical procedure suitable for overweight patients.

Alphapharm **Booth 17**

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 Australia **Booths 15 & 16**

Level 7, Avaya House, 123 Epping Rd
North Ryde NSW 2113
Ph: 03 9854 9883, Fax: 02 9870 1344
Contact: Vanessa De Los Rios
Email: vdelosri@amgen.com
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, 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.

Astrazeneca **Booth 9**

5 Alma Street
North Ryde NSW 2113
Ph: 02 9856 8003, Fax: 02 9978 3730
Contact: Elizabeth Draheim
Email: elizabeth.draheim@astrazeneca.com
Web: www.astrazeneca.com
AstraZeneca excels in providing healthcare solutions across seven therapeutic areas including cardiovascular, neuroscience, gastrointestinal, infection, oncology, pain control and anaesthesia and respiratory medicines.
AstraZeneca invests billions of dollars in research and manufacturing, spending over $16 million every working day on the research and development of medicines that meet patient needs.

Australasian Medical and Scientific Ltd **Booths 56 & 57**

Unit 2, 19-21 Gibbes St
Chatswood NSW 2067
Ph: 02 9882 3666, Fax: 02 9882 3999
Contact: Christine Merrell
Email: cmerrell@amsl.com.au
Web: www.amsl.com.au
AMSL distributes the ANIMAS Insulin pump and consumables. The Animas infusion pump is one of the smallest, fully waterproof pumps with the only colour screen. The Animas pump has the lowest basal rate (0/025 U/hr), the smallest basal increments (0.025 unit), and 0.05unit bolus dose. AMSL provides 24/7 support-line (1300_851_056).

Australian Pituitary Foundation Ltd **Booth 49**

PO Box 105
Kellyville NSW 2155
Contact: Sue Kozij
Email: support@pituitary.asn.au
Web: www.pituitary.asn.au
The APF's mission is to provide support to those who have experienced pituitary gland conditions. We promote awareness and disseminate information helpful to the medical community, public, pituitary patients and their families.
The Australian Pituitary Foundation Ltd was founded in Sydney in 1994 by pituitary patients and family members, with the endorsement of Australian health professionals who saw the need to support people who have rare conditions of the pituitary gland. In 2003 the Children's Growth Foundation merged with the APF and more recently the Foundation has been enhancing it's delivery of support and educational services to families of children affected by pituitary disorders.

Bayer Schering Pharma **Booth 59**

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Pymble NSW 2073
Ph: 02 9391 6176, Fax: 02 9391 6633
Contact: Sara George
Email: sara.george@bayerhealthcare.com
Web: www.bayer.com
Bayer is a global enterprise with core competencies in the fields of health care, nutrition and high-tech materials. The company's products and services are designed to benefit people and improve their quality of life. At the same time Bayer creates value through innovation, growth and high earning power. The Group is committed to the principles of sustainable development and to its role as a socially and ethically responsible corporate citizen. Economy, ecology and social responsibility are corporate policy objectives of equal rank.

BD Medical - Diabetes Care **Booth 58**

4 Research Park Drive
North Ryde NSW 2113
Ph: 02 8755 7000, Fax: 02 8755 7100
Contact: Michelle Dadich
Email: michelle_dadich@bd.com
Web: www.bd.com

BD Diabetes Care is focused entirely on helping make injection a better experience for every patient, every time. The result is the world's finest injection devices and a commitment to ensure that they are used properly.
BD pen needles and syringes are crafted with industry-leading technology, so patients can inject with greater comfort and ease.

Beckman Coulter **Sponsor**
Unit D, 24 College Street
Gladesville NSW 2111
Ph: 02 9844 6000, Fax: 02 9844 6096
Contact: Sylvia Johnson
Email: australianz_sales@beckman.com
Web: www.beckmancoulter.com
Beckman Coulter provides solutions that simplify, automate and innovate complex biomedical testing. Over 70% of the automated testing systems required by the modern clinical laboratory can be supplied by Beckman Coulter, including systems for Laboratory Automation, Chemistry, Immunoassay, Haematology, Haemostasis, Flow Cytometry and Molecular Diagnostics. For more information visit our website at www.beckmancoulter.com

Boehringer Ingelheim **Booths 51 & 52**
78 Waterloo Road
North Ryde NSW 2113
Ph: 02 8875 8875
Contact: Vicky Stavroulakis
Email: vicky.stavroulakis@boehringer-ingelheim.com
Web: www.boehringer-ingelheim.com
Boehringer Ingelheim is one of the world's fastest growing pharmaceutical companies, with a focus on developing new drugs and therapies in the areas of human pharmaceuticals and animal health. Our delivery of innovative medicines allows patients and consumers to realise their potential in health, life and work.

Diabetes Australia **Booth 11**
GPO Box 3156
Canberra ACT 2601
Ph: 02 6232 3815, Fax: 02 6230 1535
Contact: Karen Rocca
Email: krocca@diabetesaustralia.com.au
Web: www.diabetesaustralia.com.au/en
Diabetes Australia is the national peak body for diabetes in Australia and is committed to turning diabetes around through awareness, prevention, detection, management and cure. Through the administration of the National Diabetes Services Scheme, Diabetes Australia provides practical assistance, information and subsidised products to approximately 900,000 Australians diagnosed with diabetes.

Eli Lilly **Booth 6**
112 Wharf Road
West Ryde NSW 2114
Ph: 02 9325 4397, Fax: 02 9325 4410
Contact: Lai Leow
Email: leow_lai@lilly.com
Web: www.lilly.com
With its impressive 130 year history of developing diabetes treatments, Lilly is proud to offer health care providers and patients a suite of therapies and devices for both Type 1 and Type 2 diabetes.
This year, Lilly's diabetes portfolio has some new additions including the recent launch of pre-filled insulin pens – Humalog® KwikPen™, Humalog®Mix25 KwikPen™, and Humalog® Mix50 KwikPen™. In addition, Humalog® is indicated for use in pumps(1).
As well as its comprehensive product portfolio, Lilly continues to lead the way in understanding the impact of diabetes in Australia. Lilly has recently launched a new diabetes 'Conversation Maps' educational tool, endorsed by the IDF, designed to improve patient outcomes.
Lilly also continues to invest in research studies for both Type 1 and Type 2 diabetes as well as support educational programs such as *Directions in Diabetes, and Best Practice in Diabetes Centres* (in collaboration with the National Association of Diabetes Centres).
Lilly proudly shares your 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.
PBS Information: General benefit - treatment for Diabetes refer to PBS Schedule for full PBS listing information for each product.
(1) HUMALOG, HUMALOG MIX25, HUMALOG MIX50 MINIMUM PPRODUCT INFORMATION
Approved Indication: Treatment of diabetes mellitus. Contraindications: Hypoglycaemia; hypersensitivity to insulin lispro or one of its excipients; intravenous administration. Precautions: Any change of insulin or human insulin analogue should be made under medical supervision; loss of warning symptoms of hypoglycaemia; adjust dose for changes in exercise, diet and illness; should not be mixed with other insulins. Adverse Reactions: Hypoglycemia, allergic reactions and lipoodystrophy. Dosage: As determined by physician; subcutaneous injection; before meals (15 minutes). Refer to full PI for complete dosage information. Please review Full PI before Prescribing. Full PI is available on request from Eli Lilly. Eli Lilly Australia Pty. Limited. 112 Wharf Road, WEST RYDE NSW 2114. Based on PI last amended 14 May 2009.

Endocrine Society of Australia **Booth 50**

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 **Booth 14**

PO Box 282
North Ryde B/C NSW 1670
Ph: 02 9978 3900, Fax: 02 9889 3900
Contact: Eilis Quinn
Email: eilis.quinn@genzyme.com
Web: www.genzyme.com.au
One of the World's foremost biotechnology companies, Genzyme has a diverse portfolio of medical products that treat a variety of disorders including rare genetic, renal, transplant and immune diseases to name just a few. The oncology and endocrinology range includes the product Thyrogen, thyrotropin alfa-rch, an adjunctive agent used in the diagnostic and therapeutic management of well differentiated thyroid cancer.

GlaxoSmithKline **Sponsor**

Boronia VIC 3155
Ph: 03 9721 8748, Fax: 03 9720 2071
Contact: Greg Gear
Email: greg.i.gear@gsk.com
Web: www.gsk.com.au
GlaxoSmithKline (GSK) Australia is one of Australia's largest pharmaceutical and healthcare companies and is committed to improving the quality of human life by enabling people to do more, feel better and live longer.
GSK has four main sites in Australia, employing more than 1500 people. It is Australia's largest supplier of vaccines and a leading supplier of medicines for asthma, bacterial and viral infections, depression, migraine, gastroenterological disease, epilepsy, smoking cessation and pain relief.
The company invests up to $30 million in research and development each year, making it one of Australia's top 10 R&D companies.
We are the only pharmaceutical company to tackle the three "priority" diseases identified by the World Health Organization: HIV/AIDS, tuberculosis & Malaria

iNova Pharmaceuticals **Booth 20**

9-15 Chilvers Road
Tramleigh NSW 2120
Ph: 02 9875 6305, Fax: 02 9873 6412
Contact: Julieann Tyson
Email: j.tyson@inovapharma.com
Web: www.inovapharma.com
iNova Pharmaceutical's mission is to improve human health and well being by providing valued pharmaceutical products and services. iNova develops and markets a range of consumer healthcare and prescription medicines to Australia, New Zealand, Asia-Pacific, South Africa and other international markets. These include treatments for Weight Management (Duromine™), pain management, solar keratosis, superficial basal cell carcinoma, external genital warts, bacterial vaginosis, cardiac arrhythmias, and the consumer healthcare range, DURO-TUSS™, Difflam™, Azep® and Eyezep®
To learn more about iNova Pharmaceuticals, go to http://www.inovapharma.com

Ipsen Pty Ltd **Booth 8**

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 European pharmaceutical group.
Globally Ipsen focuses on developing highly specialised products to meet specific needs in therapeutic areas such as oncology, endocrinology and neuromuscular disorders. Ipsen has research and development facilities in Paris, London, Boston and Barcelona.
Since establishing in Australia in 2001, Ipsen has successfully launched Somatuline® Autogel®
(lanreotide) for the treatment of acromegaly and the symptoms of carcinoid syndrome, Dysport® (botulinum toxin type A) for the treatment of various neuromuscular disorders, NutropinAq® (recombinant human growth hormone), a growth hormone therapy for adults and children, and in 2009 Diphereline™ (tripterolin embonate), for the treatment of prostate cancer
Ipsen delivers customised solutions to healthcare professional and their patients according to their needs.

Medtronic Australasia **Booth 3**

97 Waterloo Rd
Ph: 02 9857 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. 3) to learn about the latest technologies in Diabetes Management.

Merck Sharp & Dohme **Booth 5**

PO Box 79
Granville NSW 2142
Ph: 02 9795 9002, Fax: 02 9795 9595
Contact: Elizabeth Bailiss
Email: elizabeth_bailiss@merck.com
Web: www.msd-australia.com.au
Merck Sharp & Dohme is a subsidiary of the global research-based pharmaceutical company Merck & Co., Inc. Since 1995 the company has brought 17 innovative new therapies to Australians – from osteoporosis and high cholesterol to antibiotics and HIV medicines. Our late stage pipeline includes vaccines for shingles, human papillomavirus rotavirus-induced infant diarrhoea; and a DP-IV inhibitor for the treatment of Type II diabetes.

Novartis Oncology **Booths 22 & 23**

54 Waterloo Rd
North Ryde NSW 2113
Ph: 02 9805 3775, 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 **Booth 4**

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, the leading supplier of insulin in Australia, and has the broadest insulin product portfolio in the industry.
Our strong commitment to changing diabetes is reflected in our focus on research and development in Australasia. Working together with Diabetes Australia, Juvenile Diabetes Research Foundation and supporting Pacific Island communities through our 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 Australia **Booth 12**

31 Victoria Avenue
Castle Hill NSW 2154
Ph: 02 9860 2222, Fax: 02 9860 2388
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 30 years of quality and innovation in diabetes care worldwide. We offer the Accu-Chek® range of: blood glucose monitors, insulin pumps, lancing devices and diabetes information management systems. Come to the Roche stand to see the exciting new Accu-Chek® products that can help your patients manage their diabetes.

Sanofi Aventis **Booth 2**

12-24 Talavera Rd
Macquarie Park NSW 2113
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Contact: Ryan Willoughby
Email: Ryan.Willoughby@sanofi-aventis.com
Web: www.sanofi-aventis.com

Sanofi-aventis, a leading global pharmaceutical company, discovers, develops and distributes therapeutic solutions to improve the lives of everyone. As a world-class R&D organisation, sanofi-aventis is developing leading positions in six major therapeutic areas: cardiometabolic diseases, thrombosis, oncology, central nervous system, internal medicine and vaccines.
With the recent acquisition of Australia's leading nutraceutical brands including Nature's Own and Cenovis, sanofi-aventis ANZ is now a horizontally integrated health care provider from complementary medicines, through to patented medicines, generics, over the counter medicines, and vaccines.
Sanofi-aventis has a strong commitment to R&D and we currently have 113 compounds in development. At sanofi-aventis we are committed to our customers, our employees, and even more importantly to the people who rely daily on our medicines.

Sapphire Bioscience Pty Ltd **Booth 13**

Suite 1, 134 Redfern Street
Redfern NSW 2016
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 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 an extensive range of antibodies, inhibitors, receptor agonists/antagonists, and transcription factor assays for metabolic and nuclear receptor research.

SciGen (Australia) Pty Ltd **Booth 25**

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 (Omnitrope and SciTropin A), 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 **Booths 26 & 27**

8 Cato Street
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Web: www.servier.com

Servier is a privately owned pharmaceutical company with a long-standing commitment to research and development, with all profit from Servier worldwide operations channelled into research and development projects.
Servier Australia's commercial interests are presently in cardiovascular disease (Coversyl - perindopril, Coversyl Plus - perindopril/indapamide and Natrilix SR 1.5mg - indapamide), diabetes (Diamicron MR - gliclazide), and most recently, postmenopausal osteoporosis (Protos - strontium ranelate).

Society of Reproductive Biology **Booth 24**

Contact: Chris Grupen
Email: cgrupen@vetsci.usyd.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 leading society for basic and applied reproductive biologists in Australasia and this year celebrates its 40th anniversary.

Solvay Pharmaceuticals **Booth 1**

Level 1, Bldg 2, 20 Bridge St
Pimble NSW 2073
Ph: 02 9440 0977, Fax: 02 9440 0910
Contact: Paul Vilner
Email: paul.vilner@solvay.com.au
Web: www.solvaypharmaceuticals.com

Solvay Pharmaceuticals is a group of companies active in more than 50 countries. Founded in 1863, it is headquartered in Brussels, Belgium with sales of over 2.7 Billion Euros in 2008 (5.4 Billion AUD). It employs over 28,000 people worldwide.
Established in Australia in 1996, Solvay focuses on 4 main Therapeutic Areas – Cardiometabolics, Neuroscience, Gastroenterology and Vaccines.
Solvay Pharmaceuticals has built a reputation in cardiometabolic research over the last 20 years, with research contributing substantially to the current treatment options available for hypertension and metabolic disorders including dyslipidemia associated with type 2 Diabetes.

Sphygmocor **Booth 53**

11/1059-1063 Victoria Road
West Ryde NSW 2114
Ph: 02 9874 8761, Fax: 02 9874 9022
Contact: Rohini Rishisen
Email: r.rishisen@atcormedical.com
Web: www.atcormedical.com
Arterial stiffness and Central Blood pressure are correlated with increased risk of cardiovascular events. Diabetic patients typically have higher Central Blood pressure and greater stiffness than non diabetic patients, putting them at greater risk. The SphygmoCor device provides validated, non-invasive measurements of both indexes. Stop by stand 53 for a free measurement.

Software for Specialists Pty Ltd **Booth 21**

PO Box 810
Fairfield NSW 1860
Ph: 1300 133 308, Fax: 02 8088 7139
Contact: Dana Segelov
Email: danasegelov@bigpond.com
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.

Avandamet®

rosiglitazone maleate/metformin HCl

see warnings

AVANDAMET (rosiglitazone maleate/metformin HCl). 2/500, 4/500, 2/1000 & 4/1000

Life-threatening lactic acidosis can occur due to accumulation of metformin. Risk factors include renal impairment, old age and the use of high doses of metformin above 2000 mg per day. The use of AVANDAMET is not recommended in patients with known ischaemic heart disease, particularly those taking nitrates. Rosiglitazone has been shown to be associated with an increased risk of myocardial ischaemic (angina, infarction) in pooled short-term clinical studies compared to combined active/placebo control (2.00% versus 1.53%, respectively), particularly in those who needed several antidiabetic drugs or nitrates. See **Precautions.**

Indications: Treatment of Type 2 diabetes mellitus as an adjunct to diet and exercise in patients inadequately controlled on metformin or rosiglitazone alone, or in patients stabilised on metformin and rosiglitazone (dual therapy)*. **Contraindications:** Hypersensitivity, diabetic ketoacidosis, pre-coma, renal failure, uncomplicated insulin regulated juvenile diabetes, diet only regulated diabetes, acute diabetic complications (metabolic acidosis, coma, infection, gangrene, surgery where insulin is essential), radiological studies using IV iodinated contrast materials, increased risk of lactic acidosis – e.g impaired renal function, cardiovascular disease, tissue hypoxia, pulmonary embolism, severe hepatic dysfunction, pancreatitis, excessive alcohol intake, concomitant use of diuretics, NYHA Class I-IV heart failure*, history of cardiac failure*, Acute Coronary Syndrome (unstable angina, NSTEMI and STEMI)*. **Precautions:** Type 1 diabetes mellitus, premenopausal anovulatory patients with insulin resistance (eg. polycystic ovary syndrome), dose-related hypoglycaemia (in triple therapy), fluid retention, congestive heart failure*, myocardial ischaemia*, peripheral artery disease*, triple oral therapy*, use with insulin, macular oedema with decreased visual acuity, bone fracture in women, lactic acidosis risk (hypoxaemia, dehydration, severe infection, trauma, surgery), renal, hepatic or cardiovascular impairment, elderly, excessive alcohol intake, vitamin B12 levels, pregnancy (Category C), lactation, children – This is not a full list, for more details refer to full PI. **Interactions:** Gemfibrozil, rifampicin, iodinated contrast media, acute alcohol intoxication. **Adverse Events:** anaemia, hypoglycaemia, flatulence, nausea, gastritis, abdominal pain, vomiting, hyperlipidaemia, diabetes mellitus aggravated, hypercholesterolaemia, weight increase, anorexia, constipation, oedema, macular oedema, heart failure*, myocardial ischaemia* bone fracture in women, – This is not a full list, for more details refer to full PI. **Dosage:** Twice daily with or just after food. For patients inadequately controlled on metformin: initiate at 4mg/day rosiglitazone plus the current dose of metformin. For patients inadequately controlled on rosiglitazone: initiate at 1000mg/day metformin plus the current dose of rosiglitazone. For patients stabilised on rosiglitazone and metformin (dual therapy): initiate at the current daily dose of rosiglitazone and metformin (divided into twice daily dosing). Rosiglitazone can be increased to 8mg/day after 6-8 weeks if greater glycaemic control is required. Metformin can be increased to 2000mg/day after 1-2 weeks if greater glycaemic control is required. *Please note change in Product Information.

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INVITED ORALS

001

ISOLATION AND CHARACTERISATION OF PORCINE ES CELLS

M. B. Nottle[1,2], I. M. Vassiliev[1,2], S. Vassilieva[1,2], L. F.S. Beebe[1,2], S. J. Harrison[1,2], S. M. McIlfatrick[1,2]

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[2]Centre for Stem Cell Research, Robinson Institute, Adelaide, SA, Australia

Embryonic stem (ES) cells have the capacity for self renewal, can remain undifferentiated in long term culture and can contribute to all the cells in the body including the germ cells. ES cells have been isolated in mice and have also been described for humans. However despite considerable effort for more than two decades ES cells which can contribute to the germline are yet to be isolated for the pig or any domestic species for that matter. We have developed a new method for isolating porcine ES cells which uses whole embryos cultured in alpha MEM with 10% serum replacement plus additives under 5% O_2. Unlike methods employed previously this method results in homogenous outgrowths whose cells resemble ES cells and which express Oct 4 and Nanog and SSEA-1(Nottle *et al*., 2009). These cells can be passaged and cryopreserved repeatedly resulting in the establishment of cell lines at similar efficiencies to that reported previously for 129Sv mice (Vassiliev I *et al.,* 2009). These cells can form embryoid bodies and can be differentiated to various cell types representative of all three germ layers (Vassiliev S *et al.,* 2009). Following their injection into blastocysts these cells localise /become incorporated in the inner cell mass and can be used to produce chimaeras when these embryos are transferred to recipient animals (I Vassiliev *et al.,* 2009). To date we have produced chimaeric pigs from one male ES cell line (Vassiliev I *et al*., 2009) . These are currently being mated to demonstrate germline transmission. Future studies will examine the applicability of our method to other species commencing with mice and cattle before extending these to humans.

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(2) Vassiliev I, Vassilieva S, Beebe LFS, Harrison SJ, McIlfatrick SM, Ashman RJ, Nottle, MB (2009) Production of chimaeric pigs using a porcine embryonic stem cell line. International Society for Ste

(3) Vassilieva S, Vassiliev I, Beebe LFS, Harrison SJ, McIlfatrick SM, Ashman RJ, Nottle MB. (2009). In vitro developmental potential of porcine embryonic stem cells. International Society for Stem Ce

002

REGULATION OF PLURIPOTENCY AND CELL CYCLE IN FETAL GERM CELLS

P. Western, J. Van Den Bergen, D. Miles, R. Ralli, A. Sinclair

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The germ cell lineage is unique in that it must ensure that the genome retains the complete developmental potential (totipotency) that supports development in the following generation. This is achieved through a number of mechanisms that prevent the early germ cell lineage from somatic differentiation and promote the capactity for functional totipotency. Part of this process involves the retained germ line expression of key genes that regulate pluripotency in embryonic stem cells, embryonic germ cells and some embryonal carcinoma cells, the stem cells of testicular tumours. Despite this, germ cells are not intrinsically pluripotent and must differentiate along the male or female pathways, a process which requires commitment of the bi-potential primordial germ cells to the spermatogenic (male) pathway and their entry into mitotic arrest, or to the oogenic pathway (females) and entry into meiosis. This involves robust regulation of regulatory networks controlling pluripotency, cell cycle and sex specific differentiation. Our work aims to further understand the mechanisms controlling differentiation, pluripotency and cell cycle in early male and female germ cells. Our data shows that mitotic arrest of male germ cells involves strict regulation of the G1-S phase check-point through the retinoblastoma protein. In addition, suppression of pluripotency in differentiating male germ cells involves post-transcriptional regulation of OCT4, transcriptional regulation of *Sox2* and *Nanog* and methylation of the *Sox2* and *Nanog* promoters. Further understanding of these processes promises to lead to a greater understanding of the molecular mechanisms underlying control of pluripotency, cell cycle and differentiation in the germ line and the initiation of germ cell derived testis tumours.

003

ENDOMETRIUM: CINDERELLA TISSUE IN A STEM CELL WORLD

C. E. Gargett

Centre for Women's Health Research/Monash Institute of Medical Research, Monash University, Melbourne, VIC, Australia

Despite human endometrium undergoing more than 400 cycles of regeneration, differentiation and shedding during a woman's reproductive years, and that in non-menstruating species (e.g. rodents) there are cycles of endometrial growth and apoptosis, endometrial stem/progenitor cells have only recently been identified. Since there are no specific stem cell markers, initial studies using functional approaches identified candidate epithelial and stromal endometrial stem/progenitor cells as colony forming cells/units (CFU) (1). Further evaluation of key stem cell properties of individual CFU demonstrated that rare $EpCAM^+$ epithelial cells and $EpCAM^-$ stromal cells underwent self renewal by

serial subcloning >3 times and underwent >30 population doublings in culture. Clonally-derived epithelial cells differentiated into cytokeratin$^+$ gland-like structures. Single stromal cells were multipotent as they differentiated into 4 mesodermal lineages; myogenic, adipogenic, osteoblastic and chondrogenic, suggesting that human endometrium contains a rare population of epithelial progenitor cells and mesenchymal stem cells (MSC) (2). Transplantation of freshly isolated human endometrial cells into immunocompromised mice reconstructed endometrial tissue that responded to estrogen and progesterone (3). Endometrial MSC can be prospectively isolated by co-expression of CD146 and PDGFRβ (4), but not Stro-1, a bone marrow MSC marker (5). Currently there are no known markers of endometrial epithelial progenitor cells. Endometrial cancer tissue harbours a small subpopulation of clonogenic, self-renewing, tumour-initiating cells, producing tumours that recapitulate parent tumours in histoarchitecture and differentiation markers (ERα, PR, cytokeratin, vimentin) when xenografted into mice, suggesting they are cancer stem cells. Candidate epithelial and stromal stem/progenitor cells have been identified in mouse endometrium as label retaining cells (LRC) in the luminal epithelium and perivascular cells at the endometrial-myometrial junction, respectively (6). It is likely that endometrial stem/progenitor cells play key roles in the development of gynecological diseases associated with abnormal endometrial proliferation such as endometriosis and endometrial cancer (7).

(1) Chan RWS et al Biol Reprod 2004;70:1738-50
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(4) Schwab KE & Gargett CE Hum Reprod 2007;22:2903-11
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(7) Gargett CE Hum Reprod Update 2007;13:87-101

004

ENDOCRINOLOGY OF ANOREXIA NERVOSA

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Massachusetts General Hospital, Boston, MA, United States

Anorexia nervosa is a psychiatric disease with a prevalence of 1-2% of college-aged women. This eating disorder is characterized by chronic starvation and complicated by severe endocrine dysregulation, including hypothalamic amenorrhea, androgen deficiency, growth hormone resistance, hypercortisolemia, and abnormalities in both appetite-regulating and enteric peptide levels. This talk will discuss the current state of our understanding of endocrine dysfunction in women with anorexia nervosa and its implications, including severe bone loss, which is observed in the majority of such women, despite young age. Available data on the efficacy and safety of investigated therapies for endocrine complications of anorexia nervosa, particularly bone loss, will also be discussed.

005

PREPARING FERTILE SOIL: THE IMPORTANCE OF ENDOMETRIAL RECEPTIVITY

L. A. Salamonsen

Uterine Biology, Prince Henry's Institute of Medical Research, Clayton, VIC, Australia

The human endometrium is receptive for implantation of a blastocyst, for only 4-5 days in each menstrual cycle. Failure of implantation is a major reason for infertility in women, and the inability to achieve endometrial receptivity is responsible for much of the failure of reproductive technologies. Endometrial receptivity requires alterations in the uterine luminal and glandular cells, particularly in terms of their secretory capacity and altered expression of adhesion molecules, along with decidualization of the endometrial stroma, which in women is initiated during the receptive phase, regardless of the presence of a blastocyst. Increased leukocyte numbers are also important. The microenvironments provided by the endometrium during the receptive phase and which support implantation are highly complex and constantly changing. The present review summarizes work from our laboratories and others, regarding these microenvironments, how they impact on receptivity and how they are disturbed in infertile women. Such microenvironments can also be manipulated to provide new contraceptive strategies for women.

006

DISORDERS OF PUBERTY

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Head, Paediatric Endocrinology and Diabetes DACS Unit, Monash Children's, Medical Centre, Clayton, VIC, Australia

Normal variation in the timing of the onset of puberty spans five to six years (8 – 14 years in girls; 9 – 14 years in boys) and this marked normal variability frequently gives rise to some challenges in deciding whether an individual child presents with a normal physiological variant or with significant underlying pathology. Secular trends towards earlier age of puberty in girls and boys are the result of the increasing prevalence of constitutional obesity. Precocious puberty is most commonly seen in girls and in the majority of cases no underlying cause is identified, however issues related to attenuated final adult height and ability to cope with menstrual hygiene at a young age need to be carefully assessed. As children with precocious puberty are mainly seen by paediatricians, it is older adolescents with pubertal delay who are more likely to present to adult endocrinologists and will be the major focus of this workshop. Pubertal delay in otherwise healthy adolescents is usually associated with either or both parents also having delayed onset of puberty. In the absence of a

positive family history, hypogonadotrophic hypogonadal pubertal delay is a frequent feature of chronic malnutrition, including anorexia nervosa, and chronic systemic disease, and pituitary pathology and syndromes such as Kallman's Syndrome should also be considered. Primary gonadal failure with hypergonadotrophic hypogonadism in girls is seen most commonly in Turner Syndrome, although autoimmune primary ovarian failure may also occur in younger girls. With the rising prevalence of constitutional obesity, polycystic ovarian syndrome as a cause of primary amenorrhoea is also being increasingly recognised. In addition to biochemical investigations in girls, a technically well performed abdominal pelvic ultrasound provides valuable information in the assessment of pubertal disorders. Issues related to hormone preparations, dosing and route of administration related to pubertal induction will also be discussed.

007

INFLAMMATORY PATHWAYS IN ENDOMETRIAL CANCER

H. N. Jabbour

MRC Human Reproductive Sciences Unit, The Queen's Medical Research Institute, Edinburgh, United Kingdom

Endometrial cancer is the most common gynaecological malignancy and accounts for 5% of cancers in women (http://info.cancerresearchuk.org/cancerstats/). The majority of endometrial cancers occur in post-menopausal women and 80% of patients are diagnosed when the tumour is confined to the uterus (stage 1 disease). Many of the risk factors for developing endometrial cancer are associated with excess exposure to oestrogen unopposed by progesterone. It is well established that local inflammatory pathways contribute to the initiation and progression of endometrial cancers via the release of local mediators to facilitate immune cell recruitment, angiogenesis and neoplastic cell proliferation and metastasis. Prostaglandins are one class of molecules that are important mediators of these processes. Prostaglandins are bioactive lipids produced from arachidonic acid by cyclooxygenase (COX) enzymes and specific terminal prostanoid synthase enzymes. Following biosynthesis, prostaglandins are rapidly transported outside the cell and exert an autocrine/paracrine function by coupling to specific prostanoid G protein-coupled receptors (GPCR).

Expression of COX enzyme, synthesis of prostaglandins and expression of various prostaglandin receptors are elevated in pathologies of the endometrium including cancer. Using genome wide array analysis, we have identified target genes that are regulated by prostaglandins such as PGF2α via interaction with its GPCR - FP receptor. Gene ontology analysis have highlighted significant elevation in expression of genes involved in inflammatory and vascular processes which are central to endometrial function. Moreover, using an array of cellular and molecular techniques and in vivo models we have established an important role for PGF2α-FP interaction in regulating inflammatory and vascular functions. To-date, suppression of the activity of prostaglandins in pathology has focussed on using inhibitors of cyclooxygenase enzymes, which are key enzymes in the pathway to prostaglandin synthesis. However, this has been associated with serious cardiovascular side effects. Development of novel drugs that target specific receptors may provide better therapeutic alternatives.

008

ANDROGEN RECEPTOR SIGNALLING IN BREAST AND PROSTATE CANCERS

W. D. Tilley

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Androgens and estrogens represent the major growth stimuli for normal prostate and breast tissues respectively, and signalling through their cognate receptors, the androgen receptor (AR) and estrogen receptor (ER), is typically enhanced in malignant tissues. A major focus of our research group is on alterations in the androgen signalling axis in both prostate and breast cancer. Our studies have provided compelling evidence that androgen signalling is a critical determinant of all stages of prostate tumorigenesis including the development of treatment resistance. W e are currently investigating the ramifications of two new findings: a single nucleotide mutation in the AR gene resulting in an AR variant (E231G) that causes prostate cancer to develop in mice, and a novel AR co-chaperone protein (SGTA) that regulates nuclear translocation in prostatic cells. We are also studying how maternal obesity may increase the risk of developing prostate cancer in later life and investigating the efficacy of novel combination therapies for prostate cancer that consist of low doses of anti-androgen and a histone deacetylase inhibitor. In contrast, our work in breast cancer indicates that the AR signalling axis is a major growth inhibitory mechanism. We have demonstrated that normal breast tissue has a high level of AR, and that lower levels (less than the median of 75% positive cells) in breast tumours confer a 4.2-fold increased risk of death. Androgens inhibit basal and estrogen-stimulated proliferation of most AR-positive breast cancer cell lines as well as normal human breast epithelial cells. This inhibitory influence is overcome by AR antagonists and medroxyprogesterone acetate (MPA), a synthetic progestin with high affinity for the AR that is commonly prescribed to women as part of hormone replacement therapy. The latter finding has important implications for the recent debate on the risk of developing breast cancer with MPA use in post-menopausal women. Finally, we have observed that AR is more highly expressed than ER in normal breast epithelial cells and that all ER-positive cells are also AR-positive. In contrast, preliminary evidence suggests that invasive breast cancers can contain tumour cells that are ER-positive but AR-negative. Collectively, these studies have formed the foundation for our current working hypothesis that AR and ER signalling represent opposing forces in normal breast tissue homeostasis. It is possible that AR signalling regulates similar molecular mechanisms in prostate and breast tissues, but with opposite effects, and that discoveries in one tissue may ultimately benefit understanding of the other.

009

KALLIKREIN-RELATED PROTEASES AS NOVEL THERAPEUTIC TARGETS IN PROSTATE AND OVARIAN CANCER

J. A. Clements[1,2], Y. Dong[2], D. Loessner[2], O. Tan[2], S. Sieh[3], J. Reichert[3], L. Burke[2], C. Stephens[1,2], M. Lawrence[1,2], S. Stansfield[1,2], J. Swedberg[1,2], A. Ramsay[1,2], J. Hooper[1,2], J. Harris[1,2], D. Hutmacher[3]

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The kallikrein-related (KLKs) peptidases are implicated in prostate and ovarian cancer invasion/metastasis via activation of growth factors, proteases and extracellular matrix degradation involved in. In our published work, we used cell biology approaches to show novel associations of KLK peptidases with processes indicative of metastasis and the potential of our novel sunflower trypsin inhibitor scaffold-engineered KLK4 inhibitor. Our current studies are directed towards discovering the precise KLK target proteins/substrates and the subsequent signalling pathways involved in these events in order to determine their therapeutic target potential. In this regard, we are using novel tissue engineered biomimetic 3D gel matrices to better mimic the *in vivo* micro-environment of prostate cancer cells especially in bone metastasis and peritoneal invasion in ovarian cancer. Pilot studies show that PC3 cells cultured on an osteoblast-derived bone matrix undergo an EMT-like change but remain dispersed on the cell surface. In contrast, LNCaP cells cluster aligning with the fibrillar structure as they invade into the bone matrix as typically seen *in vivo*. KLK4 proteolysis of the osteoblast-derived bone matrix has identified additional novel substrates. In addition, we are exploring the cell biology that underlies the reported high KLK4 or KLK7 levels associated with poorer outcome in women with epithelial ovarian cancer (EOC). Of note, KLK4 or KLK7 transfected SKOV3 EOC cells have increased chemoresistance to taxol and/or cisplastin suggesting a mechanism for this poor outcome. Furthermore, KLK7 transfected SKOV-3 cells form multicellular aggregates (MCA) in agarose suspension (a process indicative of peritoneal tumour cell spread seen in ascites fluid clinically) which can be reversed by a KLK7 blocking antibody indicating the critical role played by KLK7 in this event. These new paradigms are providing novel information on the role of KLK peptidases in prostate and ovarian cancer progression and their potential as novel therapeutic targets.

010

ESTROGENS AND THE CELL CYCLE IN BREAST CANCER

E. A. Musgrove

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Estrogen regulates cell proliferation in the normal breast and breast cancer. Cyclin D1 and c-Myc are estrogen target genes that can mimic estrogen's ability to promote cell cycle progression. They initiate pathways that are partially independent, but both activate cyclin E1-Cdk2 by sequestration and/or downregulation of the CDK inhibitor p21Waf1/Cip1, without significant increases in cyclin E1 protein levels. Cyclin E2 undergoes a marked increase in expression following estrogen treatment or inducible expression of cyclin D1, but not induction of c-Myc. This is accompanied by recruitment of E2F1 to the cyclin E2 promoter and requires the chromatin remodelling factor, CHD8. Thus cyclin E2-Cdk2 activation by estrogen occurs via E2F- and CHD8-mediated transcription of cyclin E2 downstream of cyclin D1, in contrast with the predominant regulation of cyclin E1-Cdk2 activity via CDK inhibitor association downstream of both c-Myc and cyclin D1. To gain further mechanistic insights into estrogen regulation of the cell cycle, estrogen-responsive genes were identified by transcript profiling of estrogen-treated MCF-7 breast cancer cells, and those that were also targets of c-Myc were determined. Pathway analysis based on functional annotation of the estrogen-regulated genes identified gene signatures with known or predicted roles in cell cycle control, cell growth (i.e. ribosome biogenesis and protein synthesis), cell death/survival signaling and transcriptional regulation. Overall, genes regulated following c-Myc induction accounted for half of all estrogen-regulated genes, and half of the genes in the cell cycle signature. However, 85% of genes in the cell growth signature were c-Myc-regulated, and c-Myc induction was necessary for estrogen regulation of ribosome biogenesis and protein synthesis. Thus although estrogen regulates the cell cycle by effects on both c-Myc and cyclin D1, it regulates cell growth principally via c-Myc. Overall these studies emphasise the importance of c-Myc and cyclin D1 as mediators of steroid effects on proliferation and differentation.

011

HYPOPITUITARISM

K. Miller

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An update regarding the diagnosis and treatment of hypopituitarism will be discussed during this symposium. This will include a review of etiologies, diagnostic techniques and replacement therapies utilized. In addition, an up-to-date examination of stimulation testing and pituitary hormone replacement strategies will be provided. Post-pituitary surgery and hypocortisolemia in the intensive care unit management issues will be discussed. Current guidelines regarding weight-based diagnosis of GH deficiency will be presented. Finally, data on treatment of androgen deficiency in hypopituitary women will be discussed, as will the pros and cons of androgen replacement therapy in such women.

012

THE EVOLUTION OF RADIOTHERAPY PRACTICE IN THE MODERN MANAGEMENT OF PITUITARY TUMOURS

M. Dally

Radiation Oncology, The Alfred, Melbourne, VIC, Australia

The management of patients with pituitary tumours continues to evolve in terms of radiotherapy techniques, including frameless radiosurgery and robotic patient positioning. A total of 215 patients with pituitary adenoma have been referred to the William Buckland Radiotherapy Centre (WBRC) at the Alfred Hospital since 1993. This heterogeneous group included 129 with non functioning tumours, 43 with acromegaly and 23 with Cushing's disease. Sixteen patients with prolactinoma, 2 TSH and one patient with LH/FSH secreting tumours were also seen. The median and mean follow up of those treated (183) was 37.2 and 46.5 months respectively. The median delay from initial diagnosis to radiation was 22.3 months. Sixty four patients received stereotactic radiosurgery, 89 stereotactic radiotherapy and 28 conventional radiotherapy. One patient received intensity modulated radiotherapy.

Long term complications were infrequent. One patient developed seizures associated with temporal lobe necrosis. Optic neuritis or tumour progression leading to blindness was not seen. Control over functional tumours is less clear, although normalization of GH and IGF-1 occurred for approximately 50% at a median of 40 months, for a select cohort of patients. No patient has received conventional external beam radiotherapy since 2003 nor has any patient had DXR within living memory.

013

MANAGEMENT OF CUSHING'S DISEASE

D. J. Torpy

Endocrine and Metabolic Unit, Royal Adelaide Hospital, Adelaide, SA, Australia

ACTH-dependent endogenous hypercortisolism arises from the pituitary in 65% of males and 90% of females. Despite excellent published management schema[1], management problems remain in a substantial minority of cases. Suspicion of Cushing's requires balance given its relative rarity and the shared early features with the metabolic syndrome. Reports of high rates of undiagnosed Cushing's have been tempered by recent data in larger series.[2,3] The three usual tests for hypercortisolism, 24hr urine free cortisol, 1-mg dexamethasone suppression test (DST) and the late-night salivary cortisol have crucial caveats in interpretation and application. Ectopic ACTH sources can be distinguished with the 8-mg overnight DST, CRH stimulation test and inferior petrosal sinus sampling (IPSS). Misclassification rates for these tests range from 1% (IPSS) to 8% (8-mg DST). IPSS is often required; false prediction of an ectopic source can be reduced by use of venography to identify variant venous anatomy. Pituitary MRI is limited by the 40% of cases that have no identifiable tumour and high rates of incidentalomas. Transsphenoidal surgery (TSS) has high success rates in experienced hands. Control of Cushing's is generally demonstrated by low AM cortisol levels, indicative of a suppressed HPA axis and lack of autonomous corticotropes. Glucocorticoid replacement should be based on body surface area to avoid perpetuating hypercortisolism and delaying HPA axis recovery. Uncontrolled Cushing's post-TSS can be definitively treated by reoperation, for those with evident residual but accessible tumour, or radiotherapy. Interim medical therapy with adrenal steroidogenesis inhibitors may be required. Pasireotide, the first direct corticotrope inhibitor to reach large trial status may control Cushing's[4-5]. Adrenalectomy may be needed to resolve hypercortisolism, either by laparoscopic surgery, or the adrenolytic agent mitotane. The risk of Nelson's may be overestimated in early studies but pre-ADX pituitary radiation may minimize the risk. Recurrent Cushing's post-TSS occurs in 20% over 20 years, necessitating surveillance. Overall, modern management schema are effective, but common problems include early diagnosis, true vs Pseudo-Cushing's, over-interpretation of results close to diagnostic cut-offs, invalidation of testing by intermittent or cyclic hypercortisolism, treatment of uncontrolled Cushing's post-TSS and the ongoing risk of recurrence.

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(4) Cukier K, Tewari R, Kurth F, Schmid HA, Lai C, Torpy DJ. Significant response to pasireotide (SOM230) in the treatment of a patient with persistent, refractory Cushing's disease. Clin Endocrinol (Ox

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014

PRE-TREATMENT OF ACROMEGALY AND CUSHINGS

E. Duncan

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Functional pituitary tumours require definitive management irrespective of tumour size. For acromegaly and Cushing's disease, surgery remains for most patients the treatment of choice, with its potential for cure, control of tumour size and rapid reduction in hormone secretion. However, medical management pre-surgery for these patients may improve subsequent surgical outcome, as well as improving anaesthetic risk and complications peri-procedure.

This review will discuss the evidence for medical management of acromegaly and Cushing's disease, with respect to control of tumour size, control of tumour secretion, and complications of hormonal excess.

015

A GFP COMPLEMENTATION SCREEN IN LIVING CELLS TO IDENTIFY NOVEL MODIFIERS OF GPCRS

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The peptide hormone, angiotensin II (AngII), acts via the G protein-coupled type 1 angiotensin receptor (AT1R), both peripherally and centrally, to regulate blood pressure and water and salt homeostasis. AngII also promotes cell growth and contributes to cardiovascular disease. The AT1R is widely studied as the prototypical peptide-activated G protein-coupled receptor. To define the signalling/regulatory complexes formed at the AT1R, we have utilised an assay (pioneered by Professor Stephen Michnick, Université de Montreal, Canada), termed the Protein Complementation Assay (PCA) to screen for protein-protein associations in living cells. This fluorescence-based assay relies on the concept that two protein fragments (e.g., the N- and C-terminal halves of yellow fluorescent protein (YFP), known as YFP1 and YFP2), which show no fluorescence when expressed as separate entities, produce a fluorescence signal when fused to two separate proteins that interact and bring YFP1 and YFP2 into close proximity for re-folding.

We have screened for novel AT1R interacting partners from a YFP2-cDNA library. After several rounds of screening (involving transfection of library pools and AT1R-YFP1, FACS to identify putative interactors and extraction of plasmid DNA, re-expression of candidates and finally DNA sequencing), we have identified some unique candidates, including proteins involved in: vesicular acidification, trafficking and endocytosis; post-translational modification and stability; and signalling. One of these, SUMO-1 (an ubiquitin-like protein involved in post-translational modification, location, and stability of proteins) has been confirmed by co-expression and co-immunoprecipitation as a bona fide component of the AngII-stimulated AT1R-arrestin complex.

These results show the power of unbiased screening to reveal the intricate networks that underlie intracellular communication and receptor/arrestin scaffolding and function. Such information is vital to the process of identifying appropriate targets to modify and control biology – particularly for AT1 receptors that contribute significantly to human health and disease.

016

AMYLIN RECEPTORS: A COMPLEX ROAD TO DRUG DISCOVERY

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Amylin is a 37 amino acid peptide that is co-secreted with insulin from pancreatic beta -cells following nutrient ingestion and its stable analogue, pramlintide, is currently approved for clinical treatment of Type I and II diabetes. Amylin acts to potently inhibit gastric emptying and to inhibit feeding, while at higher concentrations can promote glycogen breakdown and decrease insulin-stimulated incorporation of glucose into glycogen. Amylin is a member of the calcitonin (CT) family of peptides, which include CT, CT gene-related peptides (CGRP) and adrenomedullin (AM). The receptors for these peptides comprise the calcitonin receptor (CTR) and the CTR-like receptor (CLR) that may be complexed with one of three, single transmembrane spanning, receptor activity modifying proteins (RAMPs). Amylin receptors are formed when the CTR is in complex with RAMP1, RAMP2 or RAMP3, forming AMY1, AMY2 and AMY3 receptors, respectively. Each of these receptors, while binding amylin with similar affinity, has a distinct agonist and antagonist pharmacology. For example, the AMY1 receptor has high affinity for both amylin and CGRP, whereas the AMY3 receptor has lower affinity for CGRP. In contrast, the CTR expressed alone exhibits a phenotype with high affinity for mammalian CTs and low affinity amylin and CGRP. Analysis of RAMP chimeras and deletion constructs has provided insight into domains of RAMPs that contribute to ligand and signaling specificity. Orally active amylin mimetics are of therapeutic interest in treatment of diabetes and obesity, however, the complex nature of amylin receptors provides challenges as well as opportunities for novel drug discovery. The diffuse pharmacophore of the peptide-binding interface makes development of classical orthosteric small molecule drugs unlikely, but there is significant potential for development of novel allosteric drugs. Understanding the molecular behavior of the receptor complexes will therefore be critical to successful development of targeted therapeutics.

017

GPCR HETEROMERIZATION AND DRUG DISCOVERY

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There is now significant evidence demonstrating the capability of G protein coupled receptors (GPCRs) to interact with each other (heteromerize) either constitutively or dynamically. The next important steps are to elucidate which receptors interact, the functional consequences of these interactions and indeed the physiological relevance. However, our ability to answer these questions and open the door to drug discovery has been hampered by a lack of cell-based assays capable of systematically identifying putative heteromers or heteromer-specific compounds. To address this need, we have developed a patented cell-based assay technology that enables systematic identification of both constitutively- and dynamically-formed GPCR heteromers using a ligand-dependent reporter system. Furthermore, the GPCR Heteromer Identification Technology (GPCR-HIT) is being used to profile existing marketed drugs and carry out high throughput screens for novel drug candidates. GPCR-HIT utilizes a novel configuration of components enabling it to be performed on a number of assay platforms capable of detecting the proximity of GPCRs and β-arrestins. We have focused on developing the assay on the

bioluminescence resonance energy transfer (BRET) platform, as it is particularly well-suited to monitoring GPCR/β-arrestin interactions in live cells, in real-time (1-5). Furthermore, the potential utility of BRET for high-throughput screening has been demonstrated (1,3,5), taking advantage of the ligand-dependent nature of this interaction. BRET has been used extensively to investigate GPCR heteromerization by fusing the donor and acceptor to different GPCRs and monitoring direct interactions. A multitude of papers now claim to give evidence for GPCR heteromerization using this approach, with varying levels of rigor. Unfortunately, assessing heteromerization in this way means that specific interactions need to be differentiated from so-called 'bystander BRET', particularly if heteromers form constitutively in the endoplasmic reticulum. Our utilization of a ligand-modulated reporter system circumvents these issues, with the added benefit of enabling high-throughput screening for heteromer-selective compounds.

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018

USING GPCRS TO FUNCTIONALLY DISSECT COMPLEX NEURONAL CIRCUITS *IN VIVO*

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Within the brain neurons with a particular function or phenotype rarely occur in homogeneous, anatomically-confined groupings. Rather they are intermixed with neurons of different neurochemistry, which may, or may not have related functions. This intermixing makes the task of understanding the function of a particular neuronal type quite difficult. Such an understanding is essential for increasing our knowledge of the role of individual brain cells in generating the specific behaviors that are produced by complex circuits. Our attempt to achieve this goal is based around dissecting the cellular complexity of the rostral ventrolateral medulla (RVLM). This region, located in the brainstem, provides essential tonic drive for the generation of sympathetic vasomotor nerve activity to the vasculature. Thus it plays a pivotal role in the regulation of blood pressure. Work over the past two decades has demonstrated that there are multiple classes of neurons within the RVLM, including two types of output neurons that project from the RVLM to the sympathetic preganglionic neurons in the spinal cord (catecholaminergic (C1) and non-catecholaminergic cells); different classes of interneurons; and astrocytes. Using different combinations of replication-deficient different viruses and cell-specific promoters we have been able to target transgene expression to particular sub-groups of RVLM cells. Transgenic expression of GPCRs that couple to excitatory and inhibitory ion channels in neurons, in RVLM of the adult brain in vivo, has enabled us to begin dissecting the functions of the different cells and to understand their role in modulating blood pressure.

019

HYPONATREMIA: 2009 AND BEYOND

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Arginine vasopressin (AVP) is primarily responsible for regulating osmotic and volume homeostasis of body fluids. The AVP receptors responsible for these regulatory functions are the V_{1a} and V_2 subtypes. The V_{1a} receptors mediate vasoconstriction, glycogenolysis and platelet aggregation, while the V_2 receptors mediate renal free-water excretion and endothelial coagulation factor release. Increased AVP secretion leads to decreased free water excretion with resulting water retention. If the AVP secretion is inappropriate, it can cause a dilutional hyponatremia. Hyponatremia is the most common disorder of electrolytes encountered in clinical practice, occurring in up to 15% to 30% of both acutely and chronically hospitalized patients. Hyponatremia is important clinically because: 1) acute severe hyponatremia can cause substantial morbidity and mortality; 2) mortality is higher in hyponatremic patients with a wide range of underlying diseases; and 3) overly rapid correction of chronic hyponatremia can cause demyelination; 4) recent studies have suggested that even mild “asymptomatic” hyponatremia is accompanied by neurocognitive disturbances and gait instability. Optimal treatment strategies have not been well defined because of marked differences in symptomatology and clinical outcomes based on the acuteness or chronicity of the hyponatremia. AVP receptor antagonists have long been anticipated as a more effective method to treat hyponatremia by virtue of their unique effect to selectively increase solute-free water excretion by the kidneys (aquaresis). In 2005, the U.S. Food and Drug Administration (FDA) approved the first such agent, conivaptan, a combined V_{1a}-V_2 receptor antagonist, for short-term intravenous use, and in 2009 tolvaptan, a selective V_2 receptor antagonist, was approved for long-term oral use. Phase 3 trials indicate that both of these agents reliably reduce urine osmolality, increase electrolyte-free water excretion, and safely raise the serum sodium concentration. They are likely to become a mainstay of treatment of euvolemic and hypervolemic hyponatremia, thus heralding a new era in the management of hyponatremic disorders. This talk will describe the mechanism of action of AVP receptor antagonists, followed by a critical review the clinical data supporting the therapeutic use of AVP receptor antagonists, also known as “vaptans”, as alternatives or supplements to current therapies for the treatment of both acute and chronic hyponatremia. An algorithm for selecting appropriate therapy for hyponatremic patients based on presenting symptoms, including the use of vaptans, will be presented, and potential changes in the current treatment guidelines based on ongoing and future clinical studies will be discussed.

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020

IN VITRO MATURATION OF FARM ANIMALS OOCYTES: A USEFUL TOOL FOR INVESTIGATING THE MECHANISMS LEADING TO FULL TERM DEVELOPMENT

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Due to logistical and economical reasons assisted reproduction of domestic animals has been based mostly on the use of oocytes isolated from ovaries collected at the slaughterhouse. In order to propagate valuable or rare genetic material, perform somatic cell nuclear transfer, generate genetically modified animals it was essential to obtain fully competent oocytes that would allow full term development of the in vitro produced embryos. Such demanding need has soon made clearly evident the crucial role played by oocyte quality, how easy it is to compromise its developmental potential and the fact that it is impossible to restore it once it has been lost. Almost three decades after the first bovine, sheep, goat, horse and pig in vitro generated offsprings were born, a large body of information has accumulated on the mechanisms regulating oocyte competence and on how the latter may be preserved during all the required manipulations. The amount of knowledge is far from being complete and many laboratories are actively working to further expand it. In this review we will highlight the aspects of the ongoing research in which we have been actively involved.

021

ANDROGEN DEPRVATION THERAPY IN PROSTATE CANCER

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Prostate cancer is the most common cause of cancer in men and their second leading cause of cancer-related death after lung cancer. Each year approximately 19000 new cases are identified in Australia with 3000 annual deaths (1). Current treatment strategies are wide ranging including surveillance, surgery, radiation, and androgen deprivation therapy (ADT). Whilst surgery and radiation are the cornerstones of treatment, increasingly ADT is being employed for local disease and biochemical recurrence.

Typically gonadotrophin-releasing hormone (GnRH) agonists are used to reduce serum testosterone toward castrate levels via down-regulation of the hypothalamic GnRH receptor and suppression of pituitary gonadotrophins. Usually these medications are administered for prolonged intervals of between 6 to 30 plus months.

During this time men may experience a number of unpleasant side effects including hot flushes, lowered mood, gynaecomastia and sexual dysfunction. However more recently there is a growing appreciation that ADT has significant detrimental effects on morbidity and mortality (2,3). In particular, there appears to be an increased risk of diabetes (4,5) and premature cardiovascular disease (5,6), adverse body composition changes and reduced physical function (7,8), and accelerated bone loss with increased fracture risk (9).

Thus while ADT attempts to create a survival advantage for men with early prostate cancer or biochemical recurrence, it is incumbent upon the physician to ensure that the overall morbidity and mortality are not worsened by induction of the complications due to profound androgen deficiency. Given that greater numbers of men are undergoing ADT there is a need for critical appraisal of the current literature, development of evidenced based management guidelines and further clinical research.

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022

ADULT DNDOCRINE CONSEQUENCES OF CHILDHOOD CANCER

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Survival after childhood cancer treatment has increased significantly over 25 year, with overall >70% survival now recorded. It is estimated that within 25 years of primary diagnosis 4% of survivors will develop a second tumour with risks from 8 – 380 times expected population risk, thyroid cancer 18 times expected population rate, 50% risk of hypothyroidism and 20% risk of thyroid nodules at 20 years.

Endocrine late effects of irradiation and chemotherapy can be direct, resulting in endocrine gland hypofunction or indirect via metaplasia and malignant transformation of normal exposed tissues and via altered bone growth.

Increasing recognition of evolution of loss of endocrine function underlines a need for surveillance and planning strategies to anticipate loss and to provide solutions. Recognition of major global effects on learning, short term memory impairment and memory processing is necessary, to understand complex management needs. Beliefs related to survival and need for care may be unusual, along with risk taking behaviours.

Current information is poor, regarding precise risks for thrombotic stroke after cranial irradiation, bowel, bladder, breast and endometrial risk after local radiation. Altered timing and tempo of puberty after CXRT or total body irradiation requires in depth understanding, to provide treatment appropriate to current status. Specific losses of gonadal function vary depending on age at exposure to toxins and type of intervention. Recovery of the female ovary after chemotherapy varies, more likely after Cyclophosphamide but possible with other alkylating agents. Acquisition of optimal peak bone mass and maintenance of bone quality in adulthood is compromised by alterations in pubertal and growth cascades.

Thyroid nodularity and differentiated carcinoma is common after scatter or direct radiation, with multifocal papillary lesions and local invasion.

Future planning should involve risk-based screening and surveillance, targeted education for risk reduction and healthcare delivered by clinicians familiar with issues and risks.

023

AROMATASE INHIBITOR USE FOR PREVENTION AND TREATMENT OF ENDOCRINE SENSITIVE BREAST CANCER

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Third generation aromatase inhibitors (IAs), anastrozole (A) and letrozole (L), are superior to tamoxifen as effective adjuvant treatment for early, endocrine sensitive primary invasive breast cancer (BC) and also for extended adjuvant treatment. A, L, and Exemestane (E) are also established as effective adjuvant treatment in sequence with tamoxifen. BC recurrence is uniformly reduced compared with tamoxifen and BC mortality is reduced.

Side effects are important and generally manageable. The serious (but uncommon) tamoxifen side effects, thrombosis and uterine cancer, are not seen with the AIs and A may prevent uterine cancer. Reduction in bone density and increased fracture risk occurs, but is manageable, and appears to be confined to the period of AI treatment as fracture rates are not different to tamoxifen after treatment ceases. Other side effects, carpal tunnel syndrome, arthralgia, and hot flushes, appear to be related to risk of BC recurrence, and may be "biomarkers" of efficacy.

Current and future research is investigating much later treatment with L to reduce the 2% annual hazard rate for long term BC recurrence in women who have completed all adjuvant treatment some years earlier (LATER trial). Primary prevention with A is being investigated for postmenopausal women at increased risk. It is now established that reduction of mammography breast density by tamoxifen is both a risk and treatment efficacy biomarker for endocrine sensitive BC, and this research will be extended to AIs.

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024

CRANIAL IRRADIATION AND PITUITARY FUNCTION

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Abstract not available at time of print.

025

THE HUMAN GLUCOCORTICOID RECEPTOR B ISOFORM IN HEALTH AND DISEASE

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Glucocorticoid resistance is a serious problem that complicates the treatment of inflammatory diseases including asthma, ulcerative colitis, arthritis and hemotologic cancers. hGR α and hGR β are isoforms of the human glucocorticoid receptor that are produced by alternative splicing of the hGR pre mRNA. These receptors differ only in composition of their ligand binding domain. Historically hGR β has been thought to play a minimal role in normal human physiology since it is generally expressed at lower levels than hGR α and it does bind glucocorticoids. In this lecture I will review current knowledge of the molecular structure, regulation and physiological functions of hGR β. We show for the first time that hGR β can bind a ligand and regulate gene expression in cells. The implications of these findings strongly suggest that hGR β is a major component of glucocorticoid resistance in human disease.

026

ELUCIDATING RORALPHA FUNCTION: *INSIGHTS INTO METABOLIC DISEASE*

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Staggerer mice (*sg*/*sg*), with reduced and dysfunctional expression of the orphan nuclear receptor, ROR alpha, in all organs display a lean and dyslipidemic phenotype. Furthermore, these mice are resistant to diet induced weight gain/obesity. We investigated whether RORalpha action in skeletal muscle (a major mass peripheral lean tissue) was involved in: (i) resistance to nutritionally induced obesity; and (ii) the control of carbohydrate metabolism. We altered the RORalpha signaling cascade in skeletal muscle, by the expression of truncated RORalphaΔDE (lacking the hinge and ligand binding domain) in muscle, to understand the involvement of this contractile (and metabolic) tissue to the RORalpha phenotype. Interestingly, transgenic heterozygous mice display elevated fasting blood glucose levels and glucose intolerance. In contrast, to *sg/sg* mice with aberrant RORalpha signaling in all tissues, muscle specific attenuation of RORalpha function did not result in resistance to diet induced obesity. Expression profiling (and western analysis) identified changes in the insulin signaling cascade, and the regulation of lipid homeostasis. We suggest that selective ROR modulators may have utility in the treatment of type2 diabetes and/or obesity.

027

STEROID HORMONE RECEPTORS IN CANCER

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The ovarian hormones oestrogen and progesterone exert fundamental control over the female reproductive system by multiple independent and overlapping mechanisms. They also have non-reproductive functions in tissues such as brain and the vasculature. While aspects of their function are known through epidemiological studies and animal and cell line models, the mechanisms of their action in the human breast are poorly understood. This knowledge gap exists despite the known role of ovarian hormones in breast development and function, and in breast cancer. The causes of breast cancer are largely unknown, but the currently accepted view is that development of invasive breast cancer requires multiple genetic changes, with the original mutation occurring early in reproductive life, and additional mutations accumulating over decades until menopause, when most breast cancers are diagnosed. Although the initiating genetic mutations are largely unknown, the pivotal involvement of ovarian hormones has been known for over a century. Women without ovaries have a markedly reduced breast cancer incidence, with a risk that is analogous to the low risk of breast cancer observed in men. The underlying mechanisms by which ovarian hormones and the endocrine milieu contribute to development of breast cancer are not known. The studies to be described have used expression profiling, live cell imaging and related approaches in human tissues to elucidate the cellular targets of progesterone and the mechanisms by which the nuclear progesterone receptor regulates transcription. The results of these studies demonstrate a critical relationship between nuclear organisation and transcriptional activity in the progesterone signalling axis, and point to mechanisms by which this is disrupted in cancers. Identification of such mechanisms may lead to further understanding of critical pathways in the development and progression of breast cancer.

028

MOLECULAR MECHANISMS OF THE MINERALOCORTICOID RECEPTOR

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The mineralocorticoid receptor (MR) differs from the other steroid receptors in that it responds to two physiological ligands, aldosterone and cortisol. In epithelial tissues, aldosterone selectivity is determined by the activity of 11 b -hydroxysteroid dehydrogenase type II. In other tissues, including the heart and regions of the CNS, cortisol is the primary ligand for the MR; in some tissues it may act as an antagonist. The structural determinants of tissue and ligand-specific MR activation have yet to be identified. We have focused on interactions of the ligand-binding domain (LBD) with ligand, with the N-terminal domain and with putative co-regulatory molecules. The inability of the glucocorticoid receptor (GR) to bind aldosterone has been exploited to identify the region of the MR LBD that confers aldosterone binding. A series of MR:GR LBD chimeras has been used to narrow this to a region of 25 amino acids. Molecular modelling has been used to define the nature of this interaction.

An interaction between the N-terminus and C-terminus/LBD (N/C-interaction) observed in the MR is aldosterone-dependent and antagonised by spironolactone; unexpectedly cortisol is also an antagonist. The ability of this interaction to discriminate between other ligands has been explored.

Nuclear receptor mediated transactivation is critically dependent on, and modulated by, co-regulatory molecules. Yeast-2-hybrid kidney cDNA library screens with the MR LBD have identified proteins which exhibit a ligand-dependent interaction with the MR. Several clones have been identified that interact in the presence of either aldosterone or cortisol but not both. These differential interactions together with those observed for the N/C-interaction provide evidence of the adoption of ligand-dependent conformations by the MR LBD. The successful identification of ligand-specific interactions of the MR may provide the basis for the development of novel MR ligands with tissue specificity.

029

THE GLUCOCORTICOID RECEPTOR: ONE GENE, MANY PROTEINS – NEW MECHANISMS FOR TISSUE SPECIFIC ANTI-INFLAMMATORY ACTIONS OF GLUCOCORTICOIDS IN HEALTH AND DISEASE

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Glucocorticoids are necessary for life after birth and regulate numerous homeostatic functions in man, including glucose homeostasis, protein catabolism, skeletal growth, respiratory function, inflammation, development, behavior and apoptosis. They are also one of the most prescribed classes of anti-inflammatory drugs in the world. Our understanding of how one hormone or drug regulates all of these diverse processes is limited, although most of these actions are thought to be mediated via the glucocorticoid receptor, which is a product of a single gene. However, recent studies in our laboratory have shown that multiple glucocorticoid receptor isoforms are produced from one gene via combinations of alternative mRNA splicing and alternative translation initiation. In addition these glucocorticoid receptor isoforms are subject to several post-translational modifications including ubiquitination, phosphorylation and sumoylation which also modulate receptor function. In this lecture, we will show that these GR receptor isoforms regulate specific subsets of genes and selectively regulate distinct cellular functions such as apoptosis. In addition, we will also describe research that evaluates the role of receptor phosphorylation in regulating the diversity of genes modulated by this ligand dependent transcription factor.

030

PRIMARY ALDOSTERONISM: WHERE TO NOW?

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In 2008 the Endocrine Society published guidelines for the case detection, diagnosis and management of primary Aldosteronism. After very wide national and internatinal consultation with endocrinologists and the hypertension specialists, these guidelines are explicitly 'work-in-progress', and already have been overtaken in one important aspect by subsequently published findings. Given the prevalence of hypertension (60% of those over 50), that primary aldosteronism constitutes 10% of these subjects, and that such subjects have much higher levels of cardiovascular damage than age -, soz- and blood pressures - matched essential hypertensives. It constitutes a more and poorly recognised public health issue. In the presentation the recommendations of the guidelines will be presented and discussed, their strengths and weaknesses canvassed, and strategies to improve its management proposed.

031

HORMONES, SLEEP AND SLEEP DISORDERED BREATHING

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Hormonal secretion is both pulsatile and nycthemeral. The former allows for rapid signal exchange, whereas the latter reflects fundamental physiological processes coordinated by circadian and sleep biology. Disrupted sleep, poor sleep quality and sleep restriction are increasingly recognised to be associated with obesity, diabetes mellitus, and insulin resistance. Whether these relationships are causally related remains unproven, although preliminary data are accumulating.

Neuroendocrine networks interact with sleep disordered breathing bidirectionally. This is typified by the interaction with the hypothalamopituitary gonadal axis in men and women whereby gonadal hormones acutely alter sleep breathing and conversely, sleep disordered breathing can suppress gonadal function. Continuous positive airway pressure (CPAP) probably improves testicular function. The effect of obstructive sleep apnea on growth hormone, prolactin, adrenal and thyroidal function appears to be more modest, but available studies are largely uncontrolled (lacking obesity-matched controls for observational studies, or sham-CPAP controls for interventional studies) or small in sample size. Clinicians should be aware that sleep disordered breathing can impair neuroendocrine function and also that sleep disordered breathing is common in certain endocrine disorders such as acromegaly, Cushing disease and myxoedema. The mechanisms driving these interactions require further elucidation.

Human models (such as menopause transition, menstrual changes, androgen deficiency etc) can be used to understand the unique interplay between hormones, sleep, breathing and ageing. Interventional studies, including randomised placebo controlled trials of androgen, estrogen and progestin therapy and their impact on sleep, sleep breathing and ageing will be highlighted. The increasing use of androgen therapy in older men (as an anti-aging strategy) emphasizes the importance of understanding and publicising the potential detrimental, beneficial or neutral effects of hormonal manipulations on sleep physiology.

032

OBSTRUCTIVE SLEEP APNEA, CARDIOVASCULAR DISEASE AND METABOLIC SYNDROME

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Obstructive sleep apnea (OSA) is a disorder which is strongly associated with obesity and male gender. It causes daytime sleepiness, accidents and reduces quality of life. Population studies in several countries, including Australia, through the 1990s showed that 5-10% of adults had at least moderate OSA (apnea-hypopnea index >15 events/ hr), although prevalence is likely higher now because of the recent world-wide increase in obesity. In patients with OSA, repetitive upper airway obstructions lead to intermittent hypoxaemia, sleep fragmentation and frequent sympathetic discharges and surges in blood pressure and heart rate. OSA has been associated with oxidative stress, vascular endothelial dysfunction, insulin resistance and elevations in some inflammatory mediators. Epidemiological and clinical observational studies suggest that OSA may be an independent risk factor for stroke, myocardial infarction, atrial fibrillation and diabetes. Short term randomized controlled trials of CPAP treatment have shown small decreases in 24-hour blood pressure. Results of studies to investigate the effects of OSA treatment on inflammatory mediators and the other components of the metabolic syndrome have been less convincing largely because of inadequate sample sizes, lack of proper randomization or short duration of therapy. There are no data yet from large-scale, randomized controlled trials of OSA treatment that show improvements in hard cardiovascular endpoints. Several such studies are, however, currently in progress.

033

THE BIOLOGY OF CIRCADIAN RHYTHM DISRUPTION, SLEEP DISTURBANCE AND METABOLISM

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It is becoming apparent that our 24/7 lifestyle is a major cause of the increased incidence of metabolic and cardiovascular disorders in our society. Sleep deprivation alone has been shown to alter a range of physiological systems, but the increasing number of people engaged in shiftwork raises the most concern. There is for example, now strong evidence that shiftwork is an independent and major risk factor for weight gain, metabolic syndrome and Type 2 diabetes.

The suprachiasmatic nucleus (SCN) generates endogenous rhythmicity, is entrained by light via the retina and provides neural and hormonal signals to the rest of the body. These endogenous rhythms are generated by a suite of clock gene transcription factors. The key genes *Bmal1* and *Clock*, through interactions with period and cryptochrome genes, establish 24 hour cycles of transcription and translation in the SCN cells. In peripheral organs these same genes directly induce rhythms in other genes with diverse functions.

Shiftworkers typically are not exposed to an environment of bright light during their waking, work period and complete darkness during their sleep opportunities, like day workers. On the contrary, they live in a twilight zone of low intensity light in their workplace and sunlight during their rest period. A human who chooses to stay awake and eat and work at night will operate in major conflict with the Circadian Timing System and its coordination of various functions. However, new research has raised the possibility of reducing the level of disruption by targeting pharmacologically the genes that provide the timing signals to various organs.

We know shiftwork impairs sleep and induces fatigue with major occupational health and safety consequences, but the challenge now is to integrate diverse fields of research and develop an understanding of, and appropriate strategies to help people with disturbances to their internal rhythms.

034

MELATONIN IN THE REGULATION OF SLEEP AND ITS EFFICACY IN THE TREATMENT OF CIRCADIAN RHYTHM SLEEP DISORDERS

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The pineal hormone melatonin is synthesised and secreted mainly during the biological night, and is thought to be involved in the circadian regulation of the sleep and wake cycle. The endogenous rhythm of melatonin is closely associated with the rhythm of sleep propensity in humans. Appropriately timed melatonin administration advances the timing of human circadian rhythms, including the sleep propensity rhythm, and promotes sleep. Melatonin effects are mediated by the melatonin MT1 and MT2 receptors, although there is uncertainty about the precise role of the receptor subtypes. We have conducted a series of studies examining the effects of melatonin and melatonin agonists on sleep and circadian rhythms in protocols in which the light-dark and sleep-wake cycles were abruptly shifted. In a melatonin study, healthy participants (n=8) received either melatonin (1.5 mg) or placebo in a repeated measures design. Our studies of a dual MT1/MT2 melatonin receptor agonist, tasimelteon, used randomised, double-blind, placebo-controlled, parallel groups designs (study 1 n=39, study 2 n=412), with efficacy assessed by polysomnography. Healthy volunteers received tasimelteon (10, 20, 50 or 100 mg in study 1, and 20, 50 or 100 mg in study 2) or placebo. Melatonin and tasimelteon improve sleep and advance the timing of the circadian rhythm of sleep propensity. We suggest that these compounds may facilitate sleep during the early evening hours by effectively 'silencing' and shifting the wake maintenance zone. Melatonin and its agonists may be an effective line of treatment for sleep complaints in circadian rhythm sleep disorders, which are characterised by persistent and recurrent sleep disturbances that occur when scheduled or desired sleep times are incompatible with endogenous circadian rhythms.

035

THE ROLE OF THE SERTOLI CELL IN REGULATING SPERMATOGENESIS, IMMUNE RESPONSES AND INFLAMMATORY DISEASE: MULTIPLE FUNCTIONS, COMMON MECHANISMS?

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There is increasing evidence that the Sertoli cell, in addition to modulating responses to direct antigenic challenges (e.g. intratesticular allografts), is central to the response of the testis to inflammation and infection. Systemic inflammation exerts an inhibitory effect on spermatogenesis, which has been attributed to the effects of fever, vascular disturbances, or loss of androgenic support. However, recent studies point to more direct effects of inflammation on spermatogenesis. The discovery that Sertoli cells express Toll-like receptors (TLR), and react to TLR ligands by producing inflammatory cytokines and other mediators, provides a mechanism to account for this direct inhibition. Moreover, the pattern of cytokines produced by the Sertoli cell during inflammation is highly characteristic. For example, when stimulated with TLR ligands the Sertoli cell produces the pro-inflammatory cytokines, interleukin-1α (IL1α) and IL6, and the regulatory cytokine, activin A, but does not produce IL1β and tumour necrosis factor-α, which are major pro-inflammatory products of activated macrophages. The disruptive effects of inflammation on spermatogenesis may be attributed to the elevated production of these cytokines, all of which have stimulatory or inhibitory effects on germ cell mitosis, meiosis and apoptosis and Sertoli cell tight junction formation, In addition, activation of TLR/IL1 mediated inflammatory pathways in the Sertoli cell inhibits its ability to respond to its principal trophic hormone, follicle-stimulating hormone. Studies on the regulation of these interactions will further establish the role of the Sertoli cell in inflammation and infection. However, such studies also have important implications for normal Sertoli cell function, as TLRs can respond to endogenous ligands as well. Consequently, the Sertoli cell may be viewed as a sentinel cell, supporting and protecting spermatogenesis when conditions are optimal, but rapidly shutting down spermatogenesis in the presence of infection or illness. Intriguingly, these apparently disparate roles appear to involve common inflammation-related mechanisms.

036

SEX SPECIFIC FUNCTION OF THE HUMAN PLACENTA: IMPLICATIONS FOR FETAL GROWTH AND SURVIVAL

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The placenta plays a central role in the development of the fetus by modulating the supply of nutrients and oxygen throughout pregnancy. We have identified that the placenta adapts to the presence of a maternal pathophysiology in a sexually dimorphic manner which results in differences in fetal growth. We have reported that the female fetus reduces her growth in response to chronic maternal asthma which ensures her survival in the presence of an acute asthma exacerbation. Conversely the male fetus continues to grow normally in the presence of maternal asthma but this is associated with a poor outcome in the presence of an acute exacerbation. We propose that the sexually dimorphic response of the fetus is derived from differences in placental adaptation to a pathophysiological condition. In the presence of a female fetus and maternal asthma, we have observed global gene changes in the placenta accompanied by significant alterations in microRNA expression. Downstream of these alterations we have observed differences in protein expression especially in relation to placental cytokines and the glucocorticoid receptor. In the presence of a male fetus there are fewer changes in global placental gene and

microRNA expression, and we have observed no alterations in expression of placental cytokines or the glucocorticoid receptor. These differential adaptations ensure increased survival of the female fetus and continued growth of the male fetus in adverse conditions.

037

IMMUNE-LIKE MECHANISMS ASSOCIATED WITH OVULATION

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Ovulation is the unique biological process by which a mature oocyte and surrounding somatic cells, the cumulus cell-oocyte complex (COC), are released from the surface of the ovary into the oviduct for transport and fertilization. Ovulation is similar to an inflammatory response: the follicles become hyperemic, produce prostaglandins and synthesize a hyaluronan-rich extracellular matrix. However, this view of ovulation may be too restrictive and need to be broadened to encompass the innate immune cell surveillance response system. This hypothesis is being proposed because ovarian granulosa cells and cumulus cells express and respond to innate immune cell related surveillance proteins (Toll-like receptors 2 and 4) and cytokines such as interleukin 6 (IL6) during ovulation. In addition, recent studies indicate that the ovulation process that is set in motion by the surge of luteinizing hormone (LH) is mediated, in large part, by the EGF-like factors (Amphiregulin, epiregulin and betacellulin) and their critical activation of RAS, most probably KRAS that is expressed at high levels in granulosa cells, and the mitogen activated protein kinases, MAP3/1 (ERK1/2). Mice in which granulosa cells are depleted of ERK1/2 fail to ovulate, oocyte meiosis does not resume, COC expansion is impaired and luteinization is blocked. Thus the global molecular reprogramming of granulosa cell gene expression patterns is completely derailed. Supported, in part by NIH-HD-16229, -16272 and -07495 (SCCPIR).

038

MALE SEX HORMONES AND THE SKELETON: USING GENETICALLY MODIFIED MOUSE MODELS TO IDENTIFY THEIR MECHANISMS OF ACTION

R. A. Davey

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Androgens are essential for skeletal growth and bone accrual during puberty, and for bone maintenance post-puberty in males. The precise mechanism by which androgens exert these actions on bone however, remains unclear. It is well accepted that one of the main ways in which androgens can act on bone is *via* the androgen receptor (AR) in osteoblasts, the bone forming cells. The development of osteoblasts involves passage through three separate stages; proliferation, matrix development and maturation, and mineralisation of the bone matrix. In order to define the role of androgens via the AR at different stages of osteoblast development, we have used the Cre-loxP system, which enables tissue or time specificity of gene deletion. We have used this approach to specifically delete the AR in pre- and proliferating osteoblasts (pOBL-ARKOs), mature osteoblasts (col2.3ARKOs) and mineralizing osteoblasts (mOBL-ARKOs). By extensively characterising these genetically modified mouse models using bone histomorphometry, microCT, biochemical assays and microarray analyses, we have provided significant insight into the mechanism of androgen action in bone. We have shown that androgens act directly *via* the AR in both mature and mineralising osteoblasts to maintain trabecular bone by regulating bone resorption (1,2). Furthermore, we have demonstrated that androgens act directly *via* the AR in mineralising osteoblasts to regulate the coupling of bone matrix synthesis to mineralisation (2). We are currently extending these studies to and to investigate the role of androgens acting directly *via* the AR in pre- and proliferating osteoblasts in pOBL-ARKO mice and to identify the target genes and pathways regulated by the AR in osteoblasts. Understanding the mechanism of androgen action on bone to increase size and density and at which stage of osteoblast development androgens act, will be of great benefit in the design of new therapies for osteoporosis.

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039

SCIENTIFIC CAREER DEVELOPMENT FOR CLINICIANS AND BASIC SCIENTISTS: A BALANCE ACT BETWEEN WORK, RESEARCH, FAMILY AND FRIENDS

V. Clifton

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A successful life involves productivity and progress in your career while ensuring your friends and family don't report you as "missing in action". From a research perspective we are expected to publish papers and secure grants and fellowships regardless of whether we are basic scientists or clinician scientists. There are three basic elements that assist in building a career while continuing to have a life: publications, experimental design and time management. Publications ensure future funding and must be a priority at all times from the beginning of your PhD through to your retirement. It is important to be opportunistic with your experimental or study design so that you generate several papers from each study. Never do a study that will not result in a publication. Design experiments that encompass

opportunities to collaborate and expand your research potential. Lastly, time management is essential to balance all aspects of your life. Try to allocate blocks of time to a particular task and focus on it until it is done. Limit your weekend work so you are with friends and family. These three essential elements for career development will be discussed in the workshop.

040

KEYS TO DELIVERING A POLISHED PRESENTATION

T. Kay

St Vincent's Institute, Fitzroy, VIC, Australia

Communication of results is a vital part of good science. Giving a talk is a tremendous opportunity to bring your hard work to the attention of your colleagues – to tell them what you have found out. You are rightly proud of your findings so it is very important to do them justice and ensure that the audience "gets" what you have achieved and its significance. Giving a talk requires meaningful, well organized and presented data as well as observation of some principles of public speaking. Some of the Do's and Don'ts of good talks will be canvassed.

041

MANAGING A RESEARCH LAB: MAKING YOUR CAREER FUN AND PRODUCTIVE

K. Loveland

ARC CBD and Monash University, Clayton, VIC, Australia

As a cell biologist, I discovered that the transformation from PhD student to Postdoctoral Fellow and then into a Lab Head is an ill-defined "differentiation pathway"!

This situation therefore affords each of us the opportunity to create the lab environment we want to work in, as we build a team that works with us to study things that are both fascinating and important. The demands of dealing with grant-writing and onerous administrative tasks can be balanced by the satisfaction of working with smart people who see things differently and introduce us to new things. This talk will consider ways to develop a team approach to making discoveries and training research scientists that will have benefits for you, your co-workers and for the communities that support your activities.

042

WHAT TO DO IN YOUR THIRD YEAR OF ENDOCRINE TRAINING

S. McGrath

Department of Endocrinology, John Hunter Hospital, Newcastle, NSW, Australia

The third year of advanced training in endocrinology is a vital platform for the trainee to further develop their clinical acumen while still under supervision prior to entering clinical practice or a turning point to leave the wards and begin a research project which may lead to a stellar research career. Unfortunately, it is often looked upon with trepidation as trainees compete for limited positions that make entering the centralised training schemes of the various states like a walk in the park.

I will present a systematic, comprehensive and all-inclusive national review of the opportunities available for trainees in their third year of advanced training.

043

SHOULD YOU DO A RESEARCH HIGHER DEGREE?

R. Clifton-Bligh

Royal North Shore Hospital, St Leonards, NSW, Australia

Abstract not available at time of print.

044

ISSUES INVOLVED IN PRIVATE PRACTICE

H. Newnham

Department of Medicine, Alfred Hospital, Prahran, VIC, Australia

Establishing a private practice is an exciting challenge. It provides the first real opportunity for you to deliniate the type of care you wish to provide to the community. All too often insufficient effort is invested in making choices at this crucial juncture of your career with ad hoc choices being made on the basis of short term availability of resources and patients. Poor decisions at this stage can have long term consequences for your patients, referring GPs, your career goals, family and lifestyle. Some factors to consider include: long term career goals, type of practice, site, structure (solo/group, principal/subtenant), office systems, out of hours/leave cover, business plan, administrative responsibilities, financing and advertising. These issues will all be discussed during my presentation. Doing some locums before initiating your own practice is a good way to learn about different styles of practice.

045

ROLE OF MYOKINES IN METABOLIC REGULATION

M. A. Febbraio

Cellular & Molecular Metabolism Laboratory, Baker IDI Heart and Diabetes Institute, Melbourne, VIC, Australia

Almost 50 years ago Goldstein[1] proposed the hypothesis that muscle cells possess a "humoral" component that contributes to the maintenance of glucose homeostasis during exercise. Approximately five years ago, we identified skeletal muscle as a cytokine-producing organ, demonstrating that the metabolic and physiologic effects of exercise may be mediated by muscle derived humoral factors [for review see[2]]. We have demonstrated that interleukin-6 [IL-6] was the prototypical "myokine", up-regulated by muscle contraction and released from contracting skeletal muscle, to play important roles in lipid and glucose metabolism in metabolically active tissues[3-6]. This has led to identification that ligands that activate the IL-6 receptor are a target therapy for metabolic disease[7,8]. These and other subsequntly identified myokines, such as IL-15, were made serendipitously[2], but it is likely that contracting skeletal muscle produces many myokines that positively act on the metabolism of other organs, presenting novel targets therapeutics for the treatment of obesity related diseases such as type 2 diabetes. We are currently using gene arrays and quantitative proteomic analyses to identify novel myokines that may play a biological role in energy metabolism and may aid in the development of identifying new drug targets to treat obesity related disorders. Using these technologies, we have identified several new candidate myokines that may play a role in metabolic regulation. The role of contraction-induced muscle derived secretory proteins will be the focus of this lecture.

Supported by Grants from the NHMRC and DART

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046

FUTURE PROOFING AUSTRALIA'S MAMMALIAN BIODIVERSITY USING GENOME RESOURCE BANKING AND ART: WHERE ARE WE UP TO?

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[3]Institute of Zoology, Zoological Society of London, London, United Kingdom

The establishment of a functional genome resource bank for the genetic management and future proofing of Australian native mammals sounds great in theory, but what is the reality of this idea. In order to understand the current rate of progress in this area, we will present an overview of the inherent structural and physiological limitations of non-eutherian mammalian reproduction in terms of gamete biology and ART. For the male, these include (1) an unique mode of spermatid condensation that imparts the need for major structural changes to sperm morphology during epididymal transit, (2) a lack of cysteine protamines and disulphide bonds in the sperm chromatin that predisposes the nucleus to post-thaw chromatin relaxation, (3) an extremely stable acrosome, which to date, has not been possible to experimentally react in vitro, (4) unusual lipid composition in the plasma membrane that potentially makes the sperm cell resistant to cold shock trauma and (5) the need, in some species, for extremely high concentrations of cryoprotectant, that paradoxically, appear to be cytotoxic to the spermatozoon. Female limitations include, (1) the production of a large yolky oocyte and resulting embryo, making it difficult to cryopreserve, (2) a small and technically challenging complex reproductive tract that makes gamete recovery and artificial insemination problematic and (3) a general lack of information on marsupial reproductive physiology and behaviour that has hindered the development of protocols for timed induction of oestrus and ovulation. We shall also identify, socio-political and ethical limitations holding back the application of assisted breeding technology in these species.

047

SPERM COMPETITION, INBREEDING AND THE EVOLUTION OF SUPERIOR EJACULATES

J. Fitzpatrick

University of Western Australia, WA, Australia

The production of viable sperm is essential for male reproductive success. However, because females in many species mate with several males during a single reproductive episode, leading to sperm competition, a male's reproductive success also depends critically on the ability of his sperm to compete efficiently with those from rival males for fertilizations. Therefore, males who regularly encounter sperm competition are expected to produce high quality ejaculates. Here, I will provide an overview of how sperm morphology and performance are influenced by sperm competition, both within and between species, using recent empirical examples. Having established the importance of producing high quality ejaculates in males experiencing sperm competition, I will then examine the reproductive consequences of producing sub-optimal sperm. Given the well known role that inbreeding plays in reducing genetic quality and reproductive success, I will focus in particular on how inbreeding acts to reduce sperm quality. Finally, I will examine the consequences of inbreeding for male reproductive success in species where sperm competition is rampant. Together, these results highlight the evolutionary importance of sperm competition and inbreeding in shaping ejaculate traits.

048

FUNCTIONAL DIFFERENCES BETWEEN SEX-SORTED AND NON-SORTED SPERM

G. Evans, S. P. De Graaf, W. M.C. Maxwell

Faculty of Veterinary Science, The University of Sydney, Sydney, NSW, Australia

The development and application of flow cytometric sorting for the pre-selection of sex has progressed at an increasing rate since the first report of live pre-sexed offspring of rabbits (Johnson et al, 1989). The technique has been extended to production of pre-sexed offspring of numerous species and sorted bull semen is now widely available commercially around the world. Due to the stresses involved in the sex-sorting process, sex-sorted sperm may be functionally compromised in terms of reduced motility and viability, and their fertilising lifespan within the female reproductive tract may be reduced. Consequently, fertility *in vivo* may be compromised. However, improvements to the technology and a greater understanding of its biological impact on the sperm have facilitated recent developments in sheep, and we have demonstrated that sex-sorting is capable of selecting a functionally superior ram sperm population in terms of both *in vitro* and *in vivo* function. This has resulted in high fertility after intrauterine insemination of sex-sorted ram sperm (de Graaf et al, 2007). Unfortunately, to date, these results have not been matched in other species.

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049

INTEGRATING INFORMATIC AND MOLECULAR APPROACHES

N. Petrovsky

Flinders Medical Centre, Adelaide, SA, Australia

Modern day research presents many challenges, not least how to cope with the dramatically expanding volume of data that threat to overwhelm most scientists. Most of the blame for this flood of data can be placed on the enormous success of molecular biology that has laid bare the inner workings of the genome along with the many thousands of molecules and signaling pathways that are encoded therein. Not surprisingly, informatics has become a science in its own right and computers are now as indispensible as lab tools as pipettes. All that remains for the modern scientist to be successful is to somehow marry up all the existing data available to their particular molecule or signaling pathway of interest and thereby generate plentiful hypotheses which themselves may be initially testable *in silico* rather than having to resort to costly and time consuming wet-lab experiments. Increasingly, computer-aided modeling approaches have the potential to save time and money in the lab allowing researchers to get to the answer they are seeking more quickly. This talk will review recent major scientific and medical advances that only became possible when informatic approaches were harnessed to analyse complex molecular, genomic and clinical datasets.

050

MAKING THE MOST OF GENE ARRAY DATA: LESSONS FROM THE ER

T. Speed

Division of Bioinformatics, The Walter & Eliza Hall Institute of Medical Research, Parkville, Australia

I've been asked to talk on "making the most of gene array data" to endocrinologists.

My main experience with array data in this context is through a collaboration with Dale Leitman of UC Berkeley's Department of Nutritional Science and Toxicology, and formerly of UC San Francisco's Department of Obstetrics, Gynecology and Reproductive

Sciences. Dale studies estrogen receptor (ER) regulation and its implications for the post-menopausal state. His goal is to develop safer more selective estrogens, and in research towards that end he makes considerable use of gene expression microarrays. Using data and results from his published research, I'll illustrate some of the ways in which we have attempted to go build on gene array data, including the use of the Gene Ontology and ChIP assays, to learn more about the regulation of ERalpha and ERbeta, via various SERMS (selective estrogen receptor modulators) such as estradiol, tamoxifen and raloxifene, and other compounds.

051

APPLICATION OF TRANSCRIPT PROFILING TO THE DISCOVERY OF THE GENETIC REGULATORY NETWORKS MEDIATING DEVELOPMENT AND CARCINOGENESIS OF THE MAMMARY GLAND

C. Ormandy

Cancer Research Program, Garvan Institute of Medical Research, Darlinghurst, NSW, Australia

The development of the mammary gland is coordinated with other reproductive events via hypothalamic and fetal modulation of systemic hormones, principally estrogen and progesterone of ovarian and placental origin, and the pituitary and placental cytokines growth hormone, placental lactogen and prolactin. These hormones regulate a genomic network, composed of transcription factors and their transcriptional targets, which are responsible for developmental events such as ductal morphogenesis, the formation of lobuloalveoli and lactation. These hormones have also been implicated in breast carcinogenesis, for example top quartile serum estrogen or prolactin independently confer a greater than three-fold increase in the risk of hormone-responsive breast cancer.

Our laboratory uses Transcript Profiling of mouse models of mammary development and cancer, in combination with Transcript Profiling of human breast cancers, to define pathways involved in development and disease of the breast. Key members of these pathways will provide new prognostic and therapeutic candidates for breast cancer. This presentation will give practical examples of our application of Transcript Profiling, coupled with Meta Analysis and Gene Set Enrichment Analysis, to uncover portions of the genomic regulatory network down stream of the prolactin and estrogen receptors. This network portion may play a key role in the specification of estrogen receptor negative breast cancer and resistance to antiestrogen therapy in estrogen receptor positive cancers.

052

POLYCYSTIC OVARY SYNDROME

R. Norman

The Robinson Institute, The University of Adelaide, Adelaide, SA, Australia

Polycystic Ovary Syndrome is generally defined by the Rotterdam criteria although some doctors use the NIH consensus criteria. It is a heterogeneous condition with reproductive and metabolic manifestations and requires adequate diagnosis, investigation and management. Anovulatory infertility is common in PCOS and there are various forms of treating this including lifestyle modification, metformin, clomiphene citrate, laparoscopic ovarian drilling, ovulation induction with FSH and in vitro fertilisation. While metformin is effective for ovulation induction it has been shown to be less effective than that of clomiphene citrate.

Definition of the metabolic syndrome in PCOS is important because of the long term consequences which include an increased risk of diabetes, metabolic risk factors for cardiovascular disease and hyperlipidaemia. All patients should have these parameters assessed as part of their ongoing management.

The care of a patient with PCOS should be a collaboration between a general practitioner, an endocrinologist and gynaecologist, depending on the nature of the presentation.

053

IVF AND THE INFERTILE FEMALE

D. Healy

Departments of Obstetrics & Gynecology, Monash University, Clayton, VIC, Australia

Modern treatment of the infertile couple has focussed upon improving the following aspects of the medical care of the infertile female partner .

Delay in seeking medical care for infertility seems increasingly common. Age of first birth in Australia has passed 30 years for the first time .Societal reasons for this seem complex.

A subset of these women present at approx 40 years seeking infertility care for the first time. Other general factors such as obesity, alcohol, cigarette smoking, other recreational drug use, other prescribed medicine use should be explored on medical history.

Preconception use of folic acid & iodine is still often neglected.

Other advice, such as cervical cytology testing and rubella susceptibility, especially in migrant groups, is often neglected.

Diagnosis of chronic anovulation is important since the use of general support, and exercise and weight loss, can alone produce spontaneous ovulation and pregnancy. Clomiphene citrate use should be monitored by a day 21 measurement of serum progesterone to monitor ovulation .Metformin is also prescribed by some.

Use of FSH injections for treating clomiphene - resistant chronic anovulation appears to have become a lost art in reproductive endocrinology practice. The Monash practice will be reviewed.

IVF stimulation regimens involved clomiphene citrate, human menopausal gonadotrophin and human chorionic gonadotrophin up to 1993.The oral contraceptive pill was used increasingly after 1995 to regulate the starting time of gonadotrophin treatment. Long down-regulation with gonadotrophin hormone releasing hormone agonists were used after 2001.Recombinant FSH was used after 1994.Gonadotrophin hormone releasing hormone antagonists were used in increasing numbers of patients after 2000.Sustained follicle stimulants, fusion molecules of FSH and the carboxy terminal peptide of the human chorionic gonadotrophin subunit, became used after 2008 to allow fewer injections and more simple IVF treatment.

Damage to mothers and babies from multiple pregnancies born after ovulation induction or IVF has become a crisis. Use of ovulation induction causes more multiple pregnancies in the USA than IVF. Despite evidence that IVF single embryo transfer have better outcomes than double embryo transfers, even amongst singleton births, multiple birth rates in Europe and the USA remain at 20 -25%.

Education to prevent abuse of reproductive endocrinology treatments is needed to improve clinical outcomes for the infertile woman needing ovulation induction or IVF care.

054

MANAGEMENT OF MALE INFERTILITY

R. I. McLachlan[1]

[1]Prince Henry's Institute of Medical Research, Clayton, VIC, Australia
[2]Monash IVF, Richmond, VIC, Australia

Male reproductive dysfunction is the sole or contributory cause of infertility in half of couples. Assisted reproductive treatments (ART), particularly intracytoplasmic sperm injection (ICSI), often provide fertility however male reproductive health is not defined by the availability of motile spermatozoa. Clinicians have an obligation to evaluate and care for infertile men: natural fertility may be restorable; prevalent health issues (e.g. androgen or gonadotrophin deficiency, testicular cancer) must be sought; and the opportunity taken to improve general and sexual health.

Primary spermatogenic failure affects ~5% of men: most are unexplained (idiopathic) but increasingly genetic factors are recognised. Id entification is essential in informing couples about the prospect for normal pregnancy, transmission of infertility and/or non-gonadal disease in offspring. Karyotypic anomalies (numerical, structural) and Y chromosomal deletions are routinely assessed prior to ART. Obstruction may follow infection or vasectomy, or result from congenital absence of the vas in which cystic fibrosis gene mutations are common and demanding evaluation of female partner's gene status. Hypogonadotrophic hypogonadism (HH) is rare but replacement therapy restores fertility. Anabolic steroid-induced gonadotrophin suppression causes infertility and often protracted secondary HH upon cessation. Erectile and/or ejaculatory dysfunction of diverse aetiologies is also prevalent.

Common empirical treatments for idiopathic infertility lack placebo-controlled RCTs data. In obstruction, the choice of surgery versus sperm retrieval/ICSI is dictated by the anatomy, surgical skill and female co-factors. ICSI offers excellent fertility prospects with poor quality or testicular/epididymal sperm. In severe spermatogenic failure (even Klinefelter's), testicular biopsy allow isolation of sperm in 30-60% and their use raise issues of safety for offspring. A moderately increased rate of congenital malformations and karyotypic anomalies in IVF/ICSI offspring is described; the fertility status of ICSI-conceived boys will be the subject of intense study in the next few years. Endocrinologists and clinical geneticists have keys role in modern ART programmes.

055

NEW INSIGHTS INTO THE REGULATION OF FOOD INTAKE AND ADIPOSITY BY GHRELIN

Z. Andrews

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Ghrelin is a hormone secreted mainly from the stomach that targets the hypothalamus to drive food intake. Ghrelin will also centrally and peripherally enhance adiposity independent of food intake. Studies show that ghrelin activates food intake by stimulating AMPK. Our recent work has focused on the downstream effects of ghrelin upon AMPK stimulation in the hypothalamus. We showed that ghrelin activates mitochondrial mechanisms that enhance the bioenergetic status of cells in hypothalamus. In this talk I will discuss the important role of uncoupling protein-2 (UCP2) in potentiating ghrelin-induced food intake. In response to the acute ghrelin injection, UCP2 facilitates mitochondrial respiration, biogenesis and NPY neuronal firing. The UCP2-dependent action of ghrelin on NPY neurons is driven by a hypothalamic fatty acid oxidation pathway involving AMPK, CPT1 and free radicals, which are scavenged by UCP2. This results reveals a novel signaling modality connecting mitochondrial mediated effect on neuronal function and associated behaviour.

In additional studies we examined the long-term effect of ghrelin on adiposity in UCP2 knockout mice. UCP2 knockout mice gained significantly more weight and percent body fat compared to wild type when treated with ghrelin for 14 days. These studies reveal a peripheral role for UCP2 in fatty acid oxidation and energy metabolism. Given that ghrelin increases UCP2 in white adipose tissue, which will help to reduce accumulation of body fat, our results suggests that the role for ghrelin in energy metabolism is to prevent starvation rather than promote adiposity.

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ADIPONECTIN AND AMP-ACTIVATED PROTEIN KINASE - CHALLENGING THE DOGMA

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Adiponectin is an adipose-secreted hormone with putative anti-diabetic properties. This adipokine is reported to stimulate fatty acid oxidation in the liver and skeletal muscle, thereby reducing lipid accumulation in these tissues. Adiponectin has also been shown to suppress hepatic glucose production in association with a reduction in gluconeogenic gene expression. These effects have been mainly attributed to the ability of adiponectin to stimulate AMP-activated protein kinase (AMPK) activity in these tissues. We have found that high-molecular-weight-enriched adiponectin (HMW), AICAR and metformin rapidly suppress glucose output from primary mouse hepatocytes in close temporal relationship with their ability to activate AMPK. This acute effect (<3h) was independent of any change in gluconeogenic enzyme expression. Interestingly, all three treatments maintained their ability to suppress glucose output in hepatocytes isolated from mice lacking both α1 and α2 AMPK catalytic subunits in the liver. Similar results were obtained using hepatocytes isolated from mice that lack hepatic LKB1, the major AMPK-kinase in the liver. Thus, the effect of HMW adiponectin, metformin and AICAR to acutely suppress glucose output from hepatocytes is independent of AMPK activity. Additional studies have also demonstrated that glucose production derived mainly from glycogenolysis rather than gluconeogenesis, is also suppressed by HMW adiponectin, metformin and AICAR. These results indicate alternate, as yet undefined, mechanisms of action for adiponecin, metformin and AICAR to acutely reduce hepatic glucose production and challenge some of the basic assumptions about the mechanism of action of adiponectin.

057

BRAIN DERIVED NEUROTROPHIC FACTOR AS A POTENTIAL ANTI-OBESOGENIC DRUG TARGET

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Our group recently demonstrated that the neurotrophin, ciliary neurotrophic factor (CNTF) can increase insulin sensitivity and decrease obesity by acting on both central and peripheral tissues.[1] Another neurotrophin, namely brain derived neurotrophic factor (BDNF) is showing great promise as a factor that may contribute to the multiple health benefits associated with exercise. The notion that BDNF may play a role in energy homeostasis is not new. Animal studies have demonstrated that BDNF reduces food intake, obesity and confers hypoglycaemic action.[2, 3] Interestingly, BDNF enhances insulin signal transduction in peripheral tissues, including liver, skeletal muscle and brown adipose tissue. Despite this finding, it was originally concluded that BDNF acts exclusively through the CNS. In addition, we also demonstrated a strong association between low plasma BDNF levels and Type 2 diabetes in humans.[4]

Recent studies have demonstrated that physical exercise can increase circulating BDNF levels in healthy humans although the cellular origin of this increase is unclear. We have now demonstrated for the first time that BDNF is a contraction-inducible protein in skeletal muscle that is capable of enhancing lipid oxidation in skeletal muscle via activation of AMP-activated protein kinase (AMPK).[5]

Unfortunately, human recombinant CNTF failed when tested in clinical trials as patients developed neutralising antibodies.[6] Unlike CNTF, BDNF circulates in the plasma of humans making it, therefore, potentially more attractive compared with CNTF as an anti-obesogenic therapy. In summary, we have identified BDNF as being a novel contraction-induced muscle cell derived protein that can increase fat oxidation in skeletal muscle in an AMPK dependent fashion. Our data and that of others, therefore, raise the possibility that BDNF analogues could be used as a possible therapy to treat metabolic disease.

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PIGMENT-EPITHELIUM DERIVED FACTOR CONTRIBUTES TO METABOLIC DYSFUNCTION AND INSULIN RESISTANCE IN OBESITY

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Obesity is a major risk factor for insulin resistance and dyslipidemia; however, the factors linking these disorders are not well defined. Adipose tissue was once considered an inert energy store but is now recognised as a major endocrine organ that regulates whole body metabolism and insulin action. A recent screen of the adipocyte secretome revealed the presence of several serine protease inhibitors (serpin). Interestingly, the serpin pigment epithelium-derived factor (PEDF), was highly adundant and previous studies have reported PEDF to be upregulated in type 2 diabetes. We investigated the role of PEDF in glucose and lipid metabolism. We show that PEDF is

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predominantly released from adipocytes but is released in smaller quantities from other tissues including liver and skeletal muscle. PEDF is elevated in obese, insulin resistant mice and reduced upon weight loss and insulin sensitisation. Lean mice injected with recombinant PEDF exhibited impaired insulin sensitivity, while neutralising PEDF in obese mice enhanced insulin sensitivity. PEDF-induced insulin resistance was associated with activation of pro-inflammatory serine/threonine signalling in muscle and liver. PEDF also altered whole-body fatty acid metabolism by decreasing fatty acid oxidation in skeletal muscle, resulting in ectopic lipid deposition in muscle and liver. These biological effects were not evident in tissues of adipose triglyceride lipase-null mice. Overall, these results identify a causal role for PEDF in obesity-induced metabolic dysfunction and insulin resistance.

059

POOR GROWTH BEFORE BIRTH IMPAIRS INSULIN SECRETION – WHAT WE HAVE LEARNT ABOUT THE MECHANISMS FROM THE PLACENTALLY-RESTRICTED SHEEP

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Diabetes occurs when insulin secretion fails to increase sufficiently to compensate for developing insulin resistance. This implies that the increased risk of diabetes in adults who were small at birth reflects impaired insulin secretion as well as their well-known insulin resistance. More recently, direct evidence has been obtained that adults and children who were growth-restricted before birth secrete less insulin than they should, given their level of insulin resistance.

Our research group is using the placentally-restricted (PR) sheep to investigate the mechanisms underlying impaired insulin action (sensitivity and secretion) induced by poor growth before birth. Like the intra-uterine growth-restricted (IUGR) human, the PR sheep develops impaired insulin action by adulthood, but has enhanced insulin sensitivity in infancy, associated with neonatal catch-up growth[1, 2]. Impaired insulin action begins to develop in early postnatal life, where although basal insulin action is high due to enhanced insulin sensitivity, maximal glucose-stimulated insulin action is already impaired in males[3]. Our cellular and molecular studies have identified impaired beta-cell function rather than mass as the likely cause of impaired insulin secretion, and we have reported a novel molecular defect in the calcium channels involved in the insulin secretion pathway in the pancreas of these lambs[3]. Upregulation of IGF-II and insulin receptor are implicated as key molecular regulators of beta-cell mass in the PR lamb[3]. By adulthood, both basal and maximal insulin action are profoundly impaired in the male lamb who was growth-restricted at birth[2].

These studies suggest therapies to prevent diabetes in the individual who grew poorly before birth should target beta-cell function, possibly in addition to further increasing beta-cell mass, to improve insulin secretion capacity, and its ability to increase in response to development of insulin resistance. We are now using the PR sheep to test potential therapies, since the timing of pancreatic development and hence exposure to a growth-restricting environment, is similar to that of the human.

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OVER FEEDING EARLY IN LIFE AND RISK OF OBESITY: INSIGHT FROM THE RODENT

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While adult lifestyle factors undoubtedly contribute to the incidence of obesity and its attendant disorders, mounting evidence suggests that programming of obesity may occur following over-nutrition during development. As hypothalamic control of appetite and energy expenditure is set early in life and can be perturbed by certain exposures such as under-nutrition and altered metabolic and hormonal signals, in utero exposure to maternal obesity related changes may contribute to programming of obesity in offspring. Data from animal studies indicate both intrauterine and postnatal environments are critical determinants of the development of pathways regulating energy homeostasis. Experimental evidence in rat studies from our laboratory points to an additive detrimental impact of high fat diet consumption after weaning in animals born of obese mothers. Deleterious effects of high fat diet during pregnancy on metabolic profile, adiposity and cardiac hypertrophy were enhanced by postnatal over consumption. Even modest early postnatal overfeeding induced by litter size reduction leads to increased adiposity. Studies are needed to determine to what extent the effect of maternal and early nutritional changes persist. This presentation summarizes recent evidence of the impact of maternal obesity on subsequent obesity risk, paying particular attention to the hypothalamic regulation of appetite, and markers of metabolic control. There is an urgent need to investigate the mechanisms underlying the trans-generational effects of maternal obesity due to an extraordinary rise in the rates of maternal obesity.

061

PATERNAL FACTORS INFLUENCING GESTATIONAL OUTCOME IN OFFSPRING

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Fetal programming can often be attributed to sub-optimal, but potentially modifiable, maternal factors such as smoking and poor nutrition. Much of the literature in this field points to factors that cause intrauterine growth restriction (IUGR) and the long term consequences for offspring health. It is not greatly appreciated, however, that other complications that may occur with, or independently of, IUGR predispose offspring, and their mothers, to poor health. These include preeclampsia and gestational diabetes. Elevated maternal BMI increases the risk for most pregnancy complications. Our new data show that paternal obesity (BMI>30) and waist circumference >102cm are associated with IUGR. We have also identified polymorphisms in a number of genes that regulate how the placenta differentiates and invades the maternal decidua, and how the mother adapts to pregnancy, that are associated with adverse pregnancy outcomes. Excitingly, many of these are polymorphisms in the paternal genome. One might reasonably expect that these would be found in imprinted genes expressed only from the paternal allele. However, we have also found several non-imprinted genes in which paternal genotype has a significant influence on pregnancy outcome both on maternal and infant disease states, but also on fetal and placental growth parameters. Furthermore, these genes interact with the maternal environment including diet and smoking to profoundly affect maternal and infant health. Consequently we now propose a complicated model of the control of optimal placental and fetal growth and pregnancy outcome that includes important genetic contributions from both parents to placental genotype that regulate conceptus growth and function. Importantly, paternal genotype can influence placental gene expression and the myriad of placental hormones and growth factors secreted into the maternal circulation that modulate maternal adaptation to pregnancy and, in susceptible women, these interact with maternal genotype, BMI and lifestyle to cause poor maternal and infant health.

062

DEVELOPMENTAL ORIGINS OF TYPE 2 DIABETES: EPIGENETIC MECHANISMS IN BETA CELL FAILURE

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The abnormal intrauterine milieu of intrauterine growth retardation (IUGR) permanently alters gene expression and function of pancreatic b -cells leading to the development of diabetes in adulthood. Expression of the pancreatic homeobox transcription factor *Pdx1* is permanently reduced in IUGR and epigenetic modifications are responsible for this decrease. Exendin-4 (Ex-4), a long-acting glucagon-like peptide 1 (GLP-1) analog, given on days 1-6 of life increases *Pdx1* expression and prevents the development of diabetes in the IUGR rat. Here we show that Ex-4 increases USF-1 and PCAF association at the proximal promoter of *Pdx1*, thereby increasing histone acetyl transferase (HAT) activity leading to a permanent increase in histone H3 acetylation and H3K4 methylation. Normalization of these histone modifications precludes DNA methylation thereby preventing silencing of *Pdx1* in islets of IUGR animals. These studies demonstrate a novel mechanism whereby a short treatment course of Ex-4 in the newborn period prevents diabetes in adulthood by restoring *Pdx1* promoter chromatin structure thus preserving *Pdx1* transcription.

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MATERNAL, TRANS-GENERATIONAL AND EPIGENETIC CONTRIBUTIONS TO METABOLIC DISEASE

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Genome wide association studies have been surprising in the relatively small component of risk related to genomic variation in determining common disease. In contrast experimental and clinical evidence demonstrates mounting evidence for trans-generationally and developmentally induced effects on the individual vulnerability to disease. There is now compelling evidence that the conditions of embryonic and fetal life influence the risk of developing in later life obesity, cardiovascular disease, type two diabetes mellitus and osteoporosis. Multiple pathways are involved: some of these effects are associated with alterations in the fetal state reflecting poor maternal nutrition or increased maternal stress while others are associated with excessive energetic supplies to mother. Importantly these effects are not restricted to the extremes of the developmental environment – rather they occur across the normative range of fetal states suggesting an adaptive origin. Adapative models have been developed to explain the circumstances under which maladaptive consequences (in terms of human health) can arise from the normative processes of adaptive plasticity. Developmentally plastic mechanisms must be distinguished from developmentally disruptive processes. Plastic processes can operate for immediate advantage with longer-term trade-offs or they can operate primarily for delayed advantage. While concepts of environmentally induced developmental polyphenism are generally used only in non-mammalian development, there is evidence to suggest that the concept may also apply in mammals. The evolutionary arguments and areas of contention relating to these models will be discussed in relationship to metabolic disease. Experimental studies supported by emergent clinical observations demonstrate that these effects are mediated through epigenetic changes in both non-imprinted genes as well as imprinted genes. There are limited data to suggest both direct and indirect transmission of these effects to subsequent generations. Indirect epigenetic inheritance may be mediated by recreation of the inducing niche in each generation. Direct epigenetic inheritance has yet to be demonstrated in the human although the circumstantial evidence and evidence in other mammals is mounting. Further there is growing evidence that such effects can be prevented or reversed by potential nutritional or hormonal interventions.

064

INTRATUMORAL ESTROGEN PRODUCTION IN HUMAN BREAST CANCER - ITS REGULATION AND INHIBITION

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The majority of human breast carcinoma is estrogen-dependent, i.e., estrogen receptor present in the nuclei of tumor cells. The analysis of estrogen metabolism in postmenopausal women have lead to the importance of intratumoral estrogen production. Breast cancer converts serum or circulating adrenal androgens into estrogens in situ as a result of series of enzymatic reactions including aromatase and these locally produced estrogens work on tumor cells as an intracrine rather than endocrine manner in postmenopausal patients with breast cancer. However, human breast cancer is composed of different cell types such as carcinoma and stromal cells and it is therefore pivotal to know where the intratumoral estrogen production occur in order to obtain the better knowledge of these intracrine actions of locally produced estrogens on breast carcinoma. Estrogen production in human breast is known to be closely related to cancer development, especially stromal invasion of carcinoma cells but it is also important to know the detailed mechanisms of its induction, i.e., which factors caused excessive estrogen production in human breast tissue. An inhibition of this intratumoral estrogen production using aromatase inhibitor (AI) is currently established as the gold standard of endocrine therapy in estrogen-dependent postmenopausal patients. However, it is also true that some of those under therapy eventually develop resistance to AI treatments, which thus makes it cardinal to study the mechanisms of resistance and develop the potential alternative treatments to overcome this resistance in these patients. Therefore, in this lecture, the following three aspects of intratumoral estrogen production will be covered. 1. Where or which cell types estrogens are produced in the breast cancer tissue of patients ? 2. How intratumoral estrogen production is regulated in breast cancer ? 3. How to inhibit or suppress estrogen production in human breast cancer ?

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THE MAMMALIAN OOCYTE: FROM BENCH TO CLINIC

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The mature mammalian oocyte is the central link between generations. It is not only responsible for the transfer of the female genome between generations, but also largely determines embryo and early fetal developmental potential. For any female, oocytes are in limited supply and are easily damaged, such that the availability of high quality or developmentally competent oocytes is a fundamental rate-limiting factor in female fertility. This is particularly relevant in Australian society today with the steadily rising age to first conception which adversely affects oocyte quality and female fertility. Yet despite years of research and clinical IVF we still have a poor understanding of the molecular and cellular processes that control oocyte quality.

It is clear that oocytes acquire developmental competence in the ovarian follicle. The acquisition of competence necessitates communication between the oocyte and maternal systems, a process which endows developmental potential as the oocyte grows and matures inside the follicle. At the cellular level this is achieved by bi-directional communication between oocytes and their companion somatic cells [1]. Over the past 10 years my laboratory has focused heavily on the nature of these oocyte-somatic communication axes and their impact on oocyte quality. Over this period, our work and that of others has shaped a new paradigm in ovarian biology, which is that the oocyte is not passive in the follicle, but rather that it actively directs the differentiation of its neighbouring somatic cells into cumulus cells through the secretion of GDF9 and BMP15 growth factors [2]. In doing so, oocytes dictate the function of their neighboring cumulus cells, directing them to perform functions needed for the appropriate growth and development of the oocyte. For example, cumulus cells supply oocytes with an array of nutrients, substrates and regulatory molecules such as cAMP, many directly through gap-junctions. These communication axes establish and maintain an elaborate and intricate local oocyte-cumulus auto regulatory loop that is required to enable post-fertilisation development.

A clear clinical application of this new knowledge is in Artificial Reproductive Technologies, in particular oocyte in vitro maturation (IVM) [3]. IVM biotechnologies have the capacity to capture the vast supply of oocytes in the mammalian ovary and generate mature oocytes in vitro. Generating offspring using IVM is already a clinically and commercially viable biotechnology in livestock breeding programs, particularly in cattle. IVM is a particularly attractive technology for the treatment of human infertility, as it removes the need for expensive and potentially harmful ovarian hyperstimulation protocols used in clinical IVF. However, widespread application of IVM in humans requires an increase in efficiency and further examination of safety of the technology. Recent work from my laboratory has increased IVM success rates in animals by using GDF9 and BMP15 in IVM [2, 3] and by developing a new system of “Induced-IVM” that more closely resembles the mechanisms of oocyte maturation in vivo. Most recently, the latter approach has led to substantial increases in embryo yield and pregnancy outcomes to levels equivalent to hormone-stimulated IVF [4]. The next challenge is to adapt these new approaches to clinical/field conditions to provide new opportunities for infertile women and for the development of a wide range of reproductive biotechnologies.

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ESA ORALS

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ABSENCE OF MYOSTATIN IMPROVES CARDIAC FUNCTION FOLLOWING MYOCARDIAL INFARCT - ANIMAL MODELS

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Introduction: Myostatin is a member of the transforming growth factor- β superfamily and has a widely accepted role in skeletal muscle regulation. However, its role in cardiac myocardium remains unclear.

Objective: To determine the role of myostatin in ovine and murine cardiac muscle following myocardial infarct (MI).

Methods: Myostatin mRNA expression was measured in the peri-infarct and distal cardiac muscle in sheep at 0, 0.25, 0.5, 1, 2, 4 and 8 days (n=3/group) post-MI induced by intra-coronary injection of 45um microspheres. To determine if myostatin affects cardiac function, wild-type (C57BL/6) and myostatin-null male mice were randomised to sham-operated controls (n=6/genotype) or ligation of the coronary artery to induce MI (n>5/genotype). Cardiac function was assessed using echocardiogram at baseline and at four weeks post-MI.

Results: Myostatin mRNA expression was reduced by 97% ($p<0.01$) at one day post-infarct in the peri-infarct region of the ovine cardiac tissue. The expression was restored to that of the controls at eight days post-MI. These changes were not observed in the distal viable tissue. Preliminary ejection and shortening fractions data showed no change in cardiac function in myostatin-null mice after ligation compared to sham animals after four weeks. However, cardiac function were significantly reduced in wild-type mice at four weeks after ligation compared to sham controls ($p<0.05$).

Conclusions: We have shown that myostatin plays a role in cardiac ischaemia, both at the tissue and functional levels. We speculate that myostatin could be involved in reducing myocardium loss, regeneration of myocardium and possibly hypertrophy of the remaining viable myocardium after MI.

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ORAL IRON CHELATION INCREASES METABOLISM AND PROTECTS AGAINST DIET-INDUCED OBESITY

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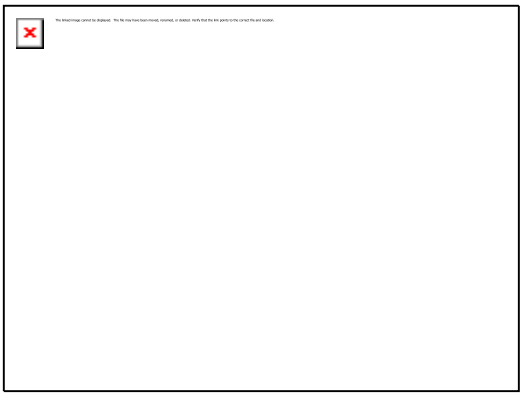
INTRODUCTION: It has been shown that orexin over-expression in mice leads to increased energy expenditure(EE) and weight loss on high fat diet (HFD) (1) . It is also suggested that orexin works via hypoxia-inducible factor(HIF)-1a (2) , a transcription factor whose activity is increased by hypoxia and iron chelation.

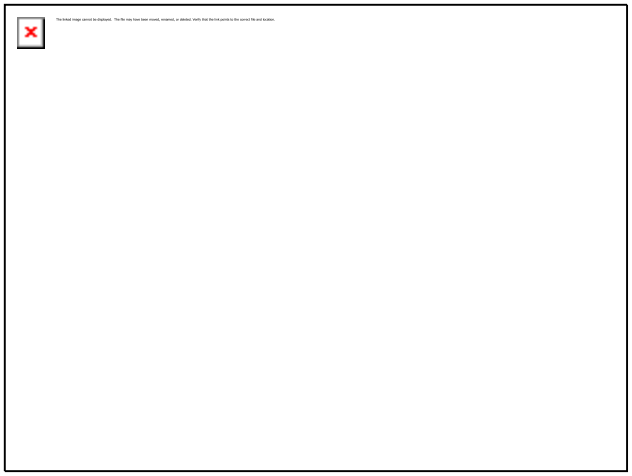
AIM: To evaluate the effects of Deferasirox (DFS), an oral iron chelator on metabolism and weight gain.

METHODS: 20 Male C57Bl/6 mice aged 6 weeks-old were fed a high fat diet (HFD) for 25 weeks. From week 0, mice were randomized to receive either DFS mixed with HFD, or control HFD. Mice were weighed weekly. Oxygen consumption (VO2), respiratory exchange ratio (RER), energy expenditure (EE) and activity were studied using the Columbus Oxymax respirator at weeks 0, 8 and 25. Food intake was quantitated over 24hrs at the same times. Rectal temperatures were measured at 2-4 weekly. Insulin tolerance test (ITT) and Glucose Tolerance Test (GTT) were performed at weeks 2 and 21 respectively. Fat depots were examined upon mice sacrifice.

RESULTS: When compared to control mice, DFS-treated mice gained less weight from week 5 onwards and by week 25, their mean weight gain almost halved that of the control mice: 9.2+/-0.8g vs 16.8+/-1.7g ($p=0.0017$). They also had improved glucose intolerance when tested at week 21. The metabolic profiles of the animals were not different at baseline but DFS-treated mice had increased VO2 and EE compared to control mice at weeks 8 and 25 (figure below). Significantly higher feed intake and rectal temperatures in DFS-treated mice were also consistent with increased metabolism as the mechanism for decreased weight gain. Upon sacrifice, DFS mice had significantly less visceral (1.4+/-0.2g vs 2.1+/-0.2g, $p=0.02$) and subcutaneous fat (0.7+/-0.2g vs 1.8+/-0.3g, $p=0.009$) depots.

CONCLUSION: Oral iron chelation increases whole body metabolism and protects against obesity and glucose intolerance in mice fed a high fat diet.





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INVESTIGATING NON-CLASSICAL SIGNALLING PATHWAYS OF THE ANDROGEN RECEPTOR

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Classical androgen receptor (AR) actions involve DNA binding and transcriptional regulation. In vitro studies suggest that non-classical AR signalling occurs in the absence of DNA binding, which involves phosphorylation of 2nd messenger cascades, including the ERK/CREB pathway. To investigate the physiological relevance of non-classical signalling, we designed an AR knockout (ARKO) mouse model (1), which expresses mutant AR that cannot bind DNA, but theoretically retains non-classical actions. Our aim is to characterise non-classical pathways in this model.

Genital skin fibroblasts (GSFs) were cultured from wildtype (WT) and ARKO males (n=3/genotype). AR binding assays were performed to determine if mutant AR can bind ligand. There was no difference between WT and ARKO in the ligand binding affinity (0.67±0.36 nM vs. 0.90±0.36 nM, mean±SEM) and the maximum ligand binding capacity (85.4±4.3 fmol 3H-dihydrotestosterone (DHT)/mg protein vs. 118.4±16.8 fmol 3H-DHT/mg protein). To demonstrate that the mutant AR retains the ability to activate non-classical pathways, WT and ARKO GSFs will be treated with DHT and 2nd messenger phosphorylation determined by Western analyses. Preliminary data show WT GSFs treated with 10 nM DHT have an increased ERK phosphorylation after 1, 5 and 30 min and we are currently investigating ARKO GSFs. We are also investigating nuclear localization and transactivation activity of the mutant AR. The AR gene in ARKO males is expressed at normal levels in kidney, fat and bone, but 6.5-fold higher than WT males in testis ($p<0.001$) and 1.5-fold higher in muscle ($p<0.05$) (quantitative real-time PCR, n=6/genotype). AR protein levels are normal in ARKO kidney, fat and bone, but 2.4-fold higher ($p<0.05$) in muscle compared to WT (Western analysis, n=4/genotype), indicating loss of autologous repression.

This study demonstrates that mutant AR, lacking the 2nd zinc finger of the DNA-binding domain, retains the ability to bind androgens, and is expressed in our ARKO model.

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SLIRP MEDIATED CROSS-TALK BETWEEN THE ANDROGEN RECEPTOR AND NOTCH SIGNALING PATHWAYS IN PROSTATE CANCER

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The androgen receptor (AR) contributes to the development and progression of prostate cancer (PCa), and even in androgen-independent PCa (AIPCa), signalling via the AR is typically maintained. The activity of nuclear receptors (NRs) such as the AR is modulated by the recruitment of coregulators (coactivators or corepressors), and altered expression and/or activity of NR coregulators is an important contributing factor for PCa relapse and progression to AIPCa. Aberrant signaling via the Notch pathway has also been identified in PCa. We recently identified a novel SRA-binding NR corepressor SLIRP, which we characterized in breast cancer, and found that it co-ordinately regulates NR activity with SKIP and SHARP, coregulators involved in both NR and Notch signalling pathways. We hypothesized that SLIRP could play an important role in both AR and Notch signalling, thus, the aim of the study was to characterise the functional role of SLIRP in each of these pathways in PCa. We found SLIRP to be widely and abundantly expressed in PCa cell lines, and in transient transfection reporter assays inhibits PSA-Luc activity. Furthermore, in chromatin immunoprecipitation studies SLIRP is

recruited to androgen-responsive promoters in PCa cells. In coculture assays, we activated the Notch pathway resulting in the activation of CBF1 and Hes1 notch responsive promoters. Transfection assays showed that SLIRP is a potent repressor of CBF1-Luc and Hes1-Luc activity. Interestingly, Hes-1 is also recruited to the PSA promoter. In summary, SLIRP participates in novel cross-talk between the AR and Notch signalling pathways acting as a potent corepressor in PCa. Binding of Hes-1 to the PSA promoter suggests an important role for members of the Notch pathway in the regulation of AR signalling in PCa.

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EXERCISE TRAINING EARLY IN LIFE PREVENTS PANCREATIC B-CELL MASS DEFICITS IN GROWTH RESTRICTED MALE RATS

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Fetal growth restriction is associated with reduced pancreatic β-cell mass that contributes to adult-onset diabetes. Exercise training enhances pancreatic morphology in diabetic animal models and has recently been shown to have long-lasting metabolic effects following exercise cessation. We therefore studied the effects of brief juvenile exercise training during a period of significant β-cell regenerative capacity in growth restricted male rats. Uteroplacental insufficiency and fetal growth restriction was induced on day 18 of pregnancy and resulted in *Restricted* litters compared to sham-operated *Controls*. To control for reduction in litter size in *Restricted*, sham-operated *Reduced litter* offspring were included, where litter size was reduced at birth, previously reported to alter postnatal lactation. Male offspring remained sedentary or underwent Early (5-9 weeks) or Late (20-24 weeks) exercise training. Exercise training involved treadmill running 5 days/week, 60 minutes/day. We examined offspring growth, islet and β-cell parameters (immunohistochemistry) and pancreatic gene expression (real-time qPCR) at 9 and 24 weeks (n=4-11). *Restricted* pups were born smaller (P<0.001) than *Controls* and remained lighter (P<0.01) until 20 weeks. *Reduced litter* were heavier (P<0.001) than *Controls* from 20 weeks. Early exercise training in *Restricted* partially normalised the 68% reduction in β-cell mass at 9 weeks, with full restoration at 24 weeks despite no further training. This reprogramming was not due to altered pancreatic Pdx-1, Vegf or Insr mRNA expression, despite being down regulated (P<0.05) as a result of uteroplacental insufficiency. Late exercise training tended to restore β-cell mass in *Restricted* but to a lesser extent. Early exercise training had beneficial effects on β-cell mass in *Controls*, but short-lived increases in *Reduced litter*. In conclusion, early life exercise training in growth restricted rats reprograms the pancreas by preventing β-cell mass deficits in adulthood . This has implications for the promotion of physical activity in low birth weight humans.

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STUDIES ON THE EPIGENETIC MECHANISMS OF REGULATION OF THE PROSTANOID RECEPTORS EP2 AND EP4 IN BREAST CANCER

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Estrogen excess is a major contributing factor to the development and progression of post-menopausal Estrogen Receptor (ER) positive breast cancers. Increased activity of Aromatase, the enzyme responsible for conversion of androgens to estrogens, and upregulation of its encoding gene CYP19A1 is often observed in breast adipose fibroblasts (BAFs) surrounding ER+ tumours. Understanding the process by which CYP19A1 is regulated between normal breast stroma and diseased tissue may help to improve outcomes for breast cancer sufferers. Prostaglandin E2 (PGE2), secreted by breast tumours, stimulates CYP19A1 expression and estrogen production in surrounding BAFs. Whilst hormonal regulation of this process has been examined, there is increasing evidence suggesting that epigenetic mechanisms, such as DNA methylation, serve to influence the PGE2 pathway. These mechanisms are thought to lead to CYP19A1 transcription by silencing or activating upstream factors. The present study aims to determine whether an inverse correlation exsisted between expression of the prostanoid receptors EP2 and EP4 and their promoter DNA methylation status in the context of (i) breast cancer derived cell lines, (ii) cancer-free BAFs and (iii) cancer-associated fibroblasts (CAFs).

Sodium bisulfite sequencing of CpG methylation within the promoter regions of EP2 and EP4 revealed differential methylation showing inverse correlation with respective mRNA expression in cell lines MDA231 (ER-), MCF7 (ER+) and MCF10F (normal epithelial). Results were confirmed with methylation-sensitive restriction enzyme analysis (msRE-PCR) and inhibition of DNA methylation with 5-aza-2'-deoxycytidine (5aza). Analysis of 9 different clinical CAF samples revealed differences in DNA methylation and mRNA expression of EP2 and EP4 compared to normal. It was found that patient ER+ status strongly correlated with a hypomethylated EP2 promoter, suggesting clinical relevance.

This study provides solid evidence for epigenetic regulation of EP2 and EP4 in breast cancer, work that may be developed further to one day develop diagnostic tests based on DNA methylation status.

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iNVESTIGATING PHYSIOLOGICAL ADAPTATIONS TO WEIGHT LOSS

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Background: In the short-term, most people are able to achieve weight loss by a change in diet or lifestyle. However, in obese people, weight loss achieved by non-surgical methods is almost always regained in the long-term. There is growing evidence that physiological adaptations to diet-induced weight loss may encourage weight regain.

Aim: To examine the effects of diet-induced weight loss on basal and post-prandial circulating levels of gastrointestinal and adipocyte hormones which influence hunger and satiety.

Design: Twenty-five non-diabetic overweight or obese (mean body mass index 35.2kg/m^2) men (n=11) and post-menopausal women (n=14) undertook a very-low-energy diet (VLED) for 8 weeks. Circulating concentrations of gastrointestinal and adipocyte hormones were examined at baseline, after 8 weeks on a VLED, and following 2 weeks of weight maintenance. Preliminary data for PYY and GLP-1 are presented here.

Results: 8 weeks on a VLED resulted in a significant reduction in mean body weight from (mean ± SEM) 100.3 ± 2.8kg to 86.7 ± 2.5kg, which was maintained during the 2-week transition to normal food (86.0 ± 2.5kg). Fasting PYY was significantly higher at baseline (75.0 ± 6.0pg/ml) than following weight loss (51.8 ± 5.8pg/ml week 8, p=0.019; 55.1 ± 5.8pg/ml week 10, p=0.051). At all post-prandial time points, PYY levels were non-significantly higher at baseline than following weight loss (p≥0.115). There were no significant differences in fasting or post-prandial GLP-1 concentrations after weight loss.

Conclusion: Diet-induced loss of 14% initial body weight resulted in reduced fasting concentrations of the satiety hormone PYY. This may be one of the mechanisms contributing to the difficulty of weight loss maintenance in obese subjects.

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DISCRETE CHANGES IN BLOOD FLOW DO NOT ELICIT TEMPERATURE EXCURSIONS IN MUSCLE TISSUE: FURTHER EVIDENCE OF A THERMOGENIC ROLE FOR MUSCLE

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Diet-induced adaptive thermogenesis accounts for up to 15% of energy expenditure in non-obese individuals. Our recent work has demonstrated post-prandial temperature excursions in skeletal muscle consistent with diet-induced thermogenesis. Given that muscle tissue comprises 30-40% of total body weight, enhancing the thermogenic potential of this tissue provides a novel means for the development of anti-obesity therapies. It is therefore imperative to determine the mechanisms responsible for post-prandial heat production. The aim of this study was to investigate the influence of regional blood flow on muscle temperature. Ovariectomised ewes (n=5, 49.2±1.4 kg) were fitted with dataloggers for temperature recordings of the vastus lateralis muscle in the hind limb. Blood flow was assessed via laser Doppler flowprobes measuring tissue flow adjacent to the datalogger (n=2), and transonic flowprobes measuring blood flow of the femoral artery (n=4). Muscle blood flow and temperature were then assessed under 3 paradigms: 1) intravenous administration of the β1/β2 adrenergic agonist, isoprenaline (0.1, 0.3, 1.0 and 3.0 μg/kg.bw), 2) post-prandial period and 3) under meal anticipation. Programmed feeding (whereby animals are fed between 1100h and 1600h) establishes post-prandial thermogenesis. Meal anticipation is invoked in a fasted animal (that has been programmed to the feeding window) by feeding animals housed with the subject. All doses of isoprenaline infusion increased blood flow to the limb. Changes in whole limb flow were consistent with discrete changes in tissue blood flow. Higher doses (1.0 and 3.0 μg) were capable of increasing muscle temperature with no further elevation in blood flow. Increases in muscle temperature were observed under both programmed feeding (0.57±0.06ºC) and meal anticipation (0.83±0.39ºC), however, neither paradigm altered blood flow to muscle tissue. These data demonstrate dissociation between muscle temperature and changes in blood flow, supporting the notion that skeletal muscle exhibits thermogenic capacity.

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CHRONIC B—BLOCKER THERAPY IS ASSOCIATED WITH BLUNTED DIET-INDUCED THERMOGENESIS, LOWER PHYSICAL ACTIVITY AND OBESITY

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The sympathetic nervous system (SNS) is a major regulator of energy metabolism and cardiovascular function. It stimulates energy expenditure and fat oxidation. Obesity has been associated with reduced SNS activity, suggesting that SNS blockade may be a risk for obesity. The aim of our study is to determine the metabolic consequences of chronic β-blocker therapy. METHODS: We evaluate β-blocker effects on energy metabolism (Study 1) and on body weight (Study 2) in a cross-sectional study design. In Study 1, resting energy

expenditure (REE), diet-induced thermogenesis (DIT) and habitual activity were compared between 10 community-dwelling β-blocker users and 20 age-, gender- and body mass index (BMI)-matched non-users. Body composition and fat distribution were measured by DEXA and abdominal CT. In Study 2, BMI and/or waist circumference were measured in outpatients with diabetes (n=219, F=87) and hypertension (n=84, F=17). RESULTS: In Study 1, REE was not different between β-blocker users and non-users. However, DIT was blunted by 50% in β-blocker users (p=0.04). Habitual activity, assessed by weekly pedometry, was reduced by 30% (p=0.03) and MET-hr/day by 35% (p=0.03). Body composition showed a trend towards increased fat mass amongst β-blocker users. In Study 2, amongst patients with diabetes, BMI was higher in β-blocker users (n=64) (33.9±6.3 vs 30.3±6.4 kg/m2, p=0.0002) than in non-users (n=150). Amongst patients with hypertension, BMI (32.7±8.6 vs 26.1±4.7 kg/m2, p=0.004) and waist circumference (109±16 vs 89±13 cm, p=0.0001) were higher in β-blocker users (n=42) than non-users (n=42). β-blocker use was an independent predictor of BMI in multiple regression analyses in both diabetic and hypertensive patients (adjusted R2=0.21 and 0.32, respectively, p<0.001). SUMMARY: DIT and habitual activity are reduced, with BMI increased in chronic β-blocker users. CONCLUSIONS: Chronic β-blockade increases the risk of obesity by blunting energy expenditure and habitual activity. (supported by NHMRC Australia)

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LATE, BUT NOT EARLY, EXERCISE RESTORES MARKERS OF MITOCHONDRIAL BIOGENESIS IN SKELETAL MUSCLE OF GROWTH RESTRICTED RATS

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Fetal growth restriction results in impaired glucose tolerance and reduced markers of skeletal muscle mitochondrial biogenesis in adulthood. Exercise training increases mitochondrial biogenesis in skeletal muscle. We therefore determined whether early or late life exercise training restored mitochondrial biogenesis markers in skeletal muscle in growth restricted male rats.

Uteroplacental insufficiency was induced on day 18 of pregnancy and resulted in *Restricted* offspring which were compared to sham-operated *Controls*. To control for reduced litter size in *Restricted*, sham-operated *Reduced litter* offspring were included, with litter size reduced at birth, shown to impair lactation and growth. Offspring remained sedentary or underwent Early (5-9 weeks) or Late (20-24 weeks) exercise training involving treadmill running 5 days/week, 60 minutes/day. We examined skeletal muscle protein expression of mitochondrial biogenesis markers (PGC1α, Tfam, CytC, COX IV and NRF2) at either 9 or 24 weeks by western blot analysis (*n*=8/group).

Restricted offspring were smaller than *Control* 1 day after birth. Rats that underwent late exercise were lighter than those who remained sedentary or underwent early exercise ($P<0.05$). Early exercise increased PGC1α protein expression at 9 weeks ($P<0.05$) but was not different between groups. *Restricted* and *Reduced litter* had lower PGC1α protein at 24 weeks compared with *Control* ($P<0.05$). Late exercise increased PGC1α protein at 24 weeks ($P<0.05$). Tfam and Cytochrome C protein was not different between groups however late exercise increased Tfam and Cyt C protein at 24 weeks ($P<0.05$). NRF2 and COX IV protein was not different.

In conclusion, early life exercise increases skeletal muscle PGC1α protein but was not sustained into adulthood after cessation of exercise. Late exercise training increased some, but not all, markers of mitochondrial biogenesis. This finding is important for growth restricted offspring who have lower PGC1α expression at 24 weeks and may contribute to preventing the onset of adult metabolic disease.

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POLYCYSTIC OVARIAN SYNDROME IN AUSTRALIAN WOMEN: RESULTS OF THE AUSTRALIAN LONGITUDINAL WOMEN'S HEALTH STUDY

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Background: Polycystic ovary syndrome (PCOS) affects 5-10% of reproductive-aged women costing ~$400 million/yr. Obesity exacerbates PCOS, the majority with PCOS are overweight, yet limited research suggests no increase in PCOS prevalence with increased BMI. The relationship between BMI and PCOS prevalence remains unresolved.

Objective: To evaluate the relationship between PCOS status and BMI in a large longitudinal study.

Design: Women aged 18-23 years at survey 1 (1996), participating in the ongoing Australian Longitudinal Study on Women's Health (ALSWH), who responded to the most recent survey (survey 4, 2006) (N=9145, 65% of the original cohort) were included (not all responded to all 4 surveys). PCOS status was based on self-reported diagnosis of PCOS. Univariate analysis was performed. Correction for confounders and regression analyses are underway.

Results: 478 women reported having PCOS (5.8%, 95% CI: 5.3%-6.4%). Except for BMI, key demographic features did not differ between PCOS and non PCOS. BMI was higher in all 4 surveys in PCOS vs non PCOS women including survey 1 (1996), (24.6 ± 5.4 vs $22.6 \pm 4.1 kg/m^2$, $p<0.0001$), and survey 4 (2006), (28.04 ± 7.2 vs $25.1 \pm 5.5 kg/m^2$, $p<0.0001$). There was a 9% increase in risk of PCOS for every unit increase in baseline BMI [Odds Ratio (95% CI): 1.09 (1.07-1.11); $p<0.0001$]. Weight gain was greater over 10 years in PCOS vs non PCOS (8.7 ± 12.7kg vs 6.4 ± 9.2kg, $p<0.0001$).

Conclusion: BMI status in all ALSWH surveys was strongly associated with PCOS diagnosis on univariate analysis. The risk of PCOS was increased with greater BMI and 10yr weight gain was greater in PCOS vs non PCOS. These novel results require further exploration, however the ALSWH data will yield important findings concerning the relationships between BMI and PCOS, predictors for weight gain and morbidities associated with greater BMI in PCOS.

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BEING BORN SMALL PROGRAMS IMPAIRED GLUCOSE TOLERANCE IN PREGNANCY

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Fetal growth restriction, induced by uteroplacental insufficiency, alters insulin sensitivity but not glucose tolerance in 6 month old females. Metabolic adaptations to pregnancy may be altered in females born small. Our aim was to investigate the effects of being born small on glucose tolerance and insulin sensitivity in non pregnant and pregnant female rats. Uteroplacental insufficiency was induced by bilateral uterine vessel ligation (Restricted) or sham surgery (Control) on day 18 of pregnancy in WKY rats. Control and Restricted non pregnant female offspring were studied at 4 months. Another cohort of Control and Restricted offspring were mated at 4 months and studied during pregnancy. Non pregnant and pregnant (day 18) females underwent an intraperitoneal glucose tolerance test (IPGTT, 1g/kg). Blood samples (plasma glucose and insulin concentrations) were collected via tail slice before and to 90 min after glucose. Post mortem was performed 2 days after IPGTT in pregnant and 2-7 days (in estrus) in non pregnant females. Restricted females were born 12-15% smaller compared to Control ($p<0.05$). Body weight and pancreas, uterine and ovarian weights were not different between Control and Restricted in non pregnant and pregnant females with no differences in maternal food intake between groups. In non pregnant females, no differences were detected in plasma glucose or insulin responses to IPGTT between groups. In pregnancy, Restricted females had a higher peak glucose (+16%) and greater area under glucose curve (+36%) compared to Control ($p<0.05$) with no differences in basal glucose or insulin concentrations. Insulin response to IPGTT and area under insulin curve was not different between groups during pregnancy. Our data demonstrates that growth restricted offspring develop impaired glucose tolerance during pregnancy but not in the non pregnant state. This suggests that the altered metabolic profile during pregnancy may influence growth and development of the next generation.

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TUMOUR-INDUCED OSTEOMALACIA: UNCOVERING THE CULPRIT

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Tumour-induced osteomalacia (TIO) is a paraneoplastic syndrome involving the secretion of phosphatonins, primarily Fibroblast Growth Factor-23 (FGF-23). It is characterised by impaired bone mineralisation secondary to renal phosphate wasting and impaired calcitriol synthesis (1). We present a case highlighting the challenges involved in the diagnosis of this rare syndrome and subsequent localisation of the underlying tumour.

A 41-year-old previously well man presented with a 15-month history of fatigue, myalgia and bone pain. Bone scintigraphy revealed multiple stress fractures of the ribs, first left metatarsal and right talar neck. Biochemical findings included markedly low serum phosphate and 25-hydroxyvitamin D levels of 0.50mmol/L and < 18nmol/L, respectively. Corrected calcium was normal. There was no clinical improvement with vitamin D repletion.

A presumptive diagnosis of TIO was made after documentation of renal phosphate wasting despite vitamin D repletion, and an elevated FGF-23 concentration of 365pg/ml (normal < 54pg/ml). Significantly, there was no family history of bone disease or hypophosphataemia. Clinical improvement occurred with phosphorus and calcitriol therapy.

Comprehensive attempts to localise the underlying tumour including a sestamibi bone study, total body magnetic resonance imaging, ^{111}In-octreotide scan and FDG-PET/CT were unsuccessful. Finally, over two years after the initial diagnosis, a 3.1cm tumour was identified in the left first intermetatarsal space with ^{68}Ga-octreotate PET/CT. This new tracer has a high affinity for somatostatin subtype-2 receptors, which have been shown to be over-expressed in tumours of patients with TIO (2).

In summary, we emphasise the importance of serum phosphate in the assessment of osteomalacia, and demonstrate the diagnostic utility of ^{68}Ga-octreotate PET/CT in TIO.

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DELAYED PUBERTY AND DIABETES INSIPIDUS IN AN ADOLESCENT MALE

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A 16-year-old male presented with reduced vision, polydipsia and polyuria, short stature and delayed puberty. Visual field testing revealed a bitemporal inferior quadrantanopia, and MRI demonstrated a supra-sellar mass. He was below the first percentile in height, weighed 47kg and was Tanner pubertal stage 1. Bone age was 13 years. Biochemical testing confirmed panhypopituitarism with diabetes insipidus.

The patient underwent trans-sphenoidal surgery. Histology showed features of a germinoma. The serum tumour markers alpha-fetoprotein and beta-human chorionic gonadotropin (βhCG) were negative, and there was a low but detectable level of βhCG in the cerebrospinal fluid. He was commenced on thyroxine, hydrocortisone and desmopressin, but testosterone therapy was postponed to optimise growth potential. Staging investigations showed no evidence of metastatic germ cell tumour. Post-operative MRI demonstrated tumour in the region of the hypothalamus, plus a second mass in the pineal gland. He was treated with radiotherapy, followed by chemotherapy with carboplatin and etoposide. The MRI post-chemotherapy showed complete resolution of the tumour.

No linear growth occurred in the eight months following the tumour diagnosis, and a testosterone-primed insulin tolerance test confirmed growth hormone and cortisol deficiency. Human growth hormone treatment was commenced, resulting in initial growth velocity of 8.4cm per year. After six months of growth hormone treatment low-dose testosterone therapy was added, with the plan to increase gradually towards adult levels. The patient's weight has increased markedly in the setting of a sedentary lifestyle and probable hypothalamic dysfunction. His height continues to increase, and he has progressed to Tanner pubertal stage 3. The latest MRI imaging demonstrated no evidence of recurrence of his germinoma.

This case highlights issues in the management of intra-cranial germ cell tumours in adolescence, including growth failure, delayed puberty and hypothalamic obesity.

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POLYURIA IN PREGNANCY

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A 32 year old pregnant lady carrying twins presented for review in the gestational diabetes clinic at 37 weeks gestation. She reported a 10 day history of polyuria and polydipsia, passing 8 – 10 litres per day. She reported nocturia every 1 – 2 hours overnight. She denied headache or visual disturbance and her blood glucose levels were well controlled on diet alone.

Physical examination revealed mild volume depletion with a blood pressure of 125/80 and a pulse rate of 92 bpm with no postural hypotension. There was no visual field deficit, with both cardiovascular and neurological examinations being unremarkable. A random blood glucose level was 6 mmol/L and urinalysis showed 3+ protein.

The patient was admitted to the obstetric ward for observation. Strict fluid balance was instituted and bloods taken. Abnormal findings included renal impairment, elevated liver enzymes (ALT and ALP), urine osmolality of 118 with serum osmolality of 295.

Urine output was monitored and found to be ~ 500 ml/hour. In light of the abnormal biochemistry and evidence of diabetes insipidus, the obstetricians proceeded to urgent caesarean section. Intravenous 5% dextrose and a brief course of ddAVP treatment were administered peripartum with a good clinical response. The diabetes insipidus and biochemical abnormalities resolved several days after delivery. Subsequent imaging of the pituitary gland showed no abnormalities.

Central and nephrogenic diabetes insipidus (DI) may manifest during pregnancy. Causes of central DI include lymphocytic hypophysitis, an enlarging pituitary mass and Langerhans cell histiocytosis. Physiological changes in salt and water homeostasis during pregnancy may unmask latent DI. Transient DI occurs in the third trimester and is linked to high levels of placental vasopressinase. This condition may be associated with preeclampsia, acute fatty liver and multiple gestation.

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ATYPICAL PITUITARY TUMOUR

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Aggressive pituitary tumours are rare, management is challenging, they respond poorly to current therapies and are often associated with a dismal prognosis (1). The incidence of true pituitary carcinoma as defined by the presence of cranio-spinal or systemic metastases is 0.2% of pituitary tumours (2). Progression to carcinoma usually occurs over a long time interval (1, 3). Reliable prognostic markers are lacking. However, in anticipation of possible aggressive behaviour, the WHO (2000) Classification of Endocrine Tumours included a third category of pituitary tumour, the "atypical" adenoma (4). We present a case of an aggressive non-functioning pituitary tumour with rapid transformation from a typical benign adenoma through to atypical adenoma in twelve months and then discovery of multiple systemic metastases most consistent with a pituitary carcinoma a further sixteen months later. The diagnostic challenge in this case has been the historical presence of a lung lesion found in 1992, some 14 years prior to the clinical presentation with pituitary tumour. Discussion will focus on the occasional difficulty in distinguishing between a pituitary carcinoma and neuroendocrine carcinoma and on the potential therapeutic role of temozolomide chemotherapy in this setting.

In addition, we present a review of the 244 archived pituitary tumour samples available within the department of Anatomical Pathology at RNSH between 2000 and 2009, of which seven were atypical tumours, three meeting the criteria for pituitary carcinoma. This represents 3.2% of resected pituitary tumours in our institution. The pathological and clinical findings of these cases will be presented.

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V EXCESS AND K DEPLETION

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Case 1 is a 19yo male presenting with four months of increasing weakness, polydipsia, polyuria, diarrhoea, postural dizziness and weight loss. His weight decreased from 90kg to 55kg over eighteen months with severe dietary restriction and daily duramine and laxative abuse until 2008. He now drinks 2 litres of 'Pepsi' and 'V' daily.

Electrolytes(mmol/L)- K 2.5, Na 137, Cl 84, HCO3 40, Cr 70, Ca(corr) 2.49, Mg 0.92, PO4 0.69

FBGL 3.8, pH 7.49, Spot urine pH 9, K 68, Na 183, diuretic/laxative screen Neg, ECG U waves

Renin 8.1ng/ml(0.2-2.8), Aldosterone 268(30-450), TSH/Cortisol/ACTH/Coeliac serology normal 24h urine collection (post-potassium replacement).

- Volume 1010ml, K 10mmol/d (40-100), Ca 11mmol/d (2.5-6.2), Mg 6.7mmol/d (5.0-7.0)

Serum potassium improved to 4.1mmol/L with 130mmol intravenous KCl over 18 hours and subsequently stabilised with reduced caffeine consumption and 1200mg potassium chloride daily.

Case 2 is a 24yo male with poorly controlled Type 1 Diabetes who presented with refractory hypokalaemia and diabetic ketoacidosis, due to insulin omission during a febrile illness. Past history included hypokalaemia and high caffeine consumption, with 3.9L 'V' and 5 cups of coffee daily. Electrolytes(mmol/L)- K 2.5, Na 134, Cl 108, HCO3 7.0, Cr 80, Ca(corr) 2.07, Mg 0.52, PO4 0.24, glucose 18.0, pH 7.24, Spot urine pH 5, ketones ++, diuretic screen Neg TSH 0.65mIU/L(0.5-4.0), Renin 5.5ng/ml, Aldosterone 449 24h urine: 1) In ICU during resuscitation & K infusion, 2) Repeat on 2xcoffee/d & K supplement - 1) volume 10556ml, K 443mmol/D - 2) volume 2208ml, pH 6.5, K 33mmol/D, Ca 1.5mmol/D, Mg 1.3mmol/D Serum potassium improved from 2.5mmol/L to 3.1mmol/L with 120mmol intravenous and 1644mg oral potassium replacement over 12 hours. Post-discharge, it was 3.9mmol/L on 1200mg/D potassium chloride with reduced caffeine consumption.

These cases describe hypokalaemia of unclear aetiology, however both patients consumed >360mg caffeine per day. Both responded to caffeine reduction.

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A CASE OF ACTH-INDEPENDENT CUSHING'S SYNDROME WITH BILATERAL ADRENAL ADENOMAS

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ACTH-independent Cushing's syndrome usually occurs in the context of a unilateral adrenocorticoid adenoma. We recently encountered a patient with ACTH-independent Cushing's syndrome and bilateral adrenal adenomas.

The patient was a 50 year-old woman with a history of osteoporotic fractures, and, particularly over the past 2 years, weight gain, depression, insomnia, lethargy and easy bruising.

Her past medical history included hypertension, hyperlipidemia, fatty liver disease and removal of an interscapular fat pad 3 years previously. Clinically, she had features of Cushing's syndrome which was confirmed by elevated 24 h urinary cortisol (490 nmol/d NR:50-250) and persistent elevation of serum cortisol (533 nmol/L) after a 1.0 mg dexamethasone suppression test.

As the ACTH level was undetectable, she had an abdominal CT scan which demonstrated bilateral adrenal adenomas measuring 2.8 cm (right) and 1.3 cm (left). Adrenal venous sampling (AVS) performed under 8mg of dexamethasone suppression demonstrated predominant cortisol secretion from the right adrenal. However, both adrenals produced cortisol autonomously, with right and left adrenal vein to peripheral vein ratios of 20:1 and 1.75:1, respectively.

The patient underwent a right partial laparoscopic adrenalectomy and histology confirmed a "benign cortical adenoma". She received standard anticoagulation postoperatively and was discharged on day 5 post-operatively but represented 3 weeks after the surgery with extensive bilateral pulmonary emboli. At 6 months post-operatively, she has low endogenous cortisol levels (30 nmol/L) and still requires cortisone replacement.

Our case highlights the added complexity of investigating ACTH-independent Cushing's syndrome with bilateral adrenal pathologies. While AVS in our case demonstrated predominant secretion from the right adrenal, the small degree of autonomous cortisol secretion on the left may represent an early "functioning" cortisol producing adenoma.

Furthermore, Cushing's syndrome is a hypercoagulable state due to an increase in coagulation factors, which persists up to 12 months post-operatively. This raises the issue of whether prolonged prophylactic anticoagulation should be considered post-operatively.

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THE BIOLOGICAL ROLE OF THE CALCITONIN RECEPTOR TO PROTECT AGAINST INDUCED HYPERCALCAEMIA IS MEDIATED PREDOMINANTLY VIA ITS ACTIONS ON OSTEOCLASTS, THE BONE RESORBING CELLS

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Calcitonin (CT) acting via its receptor, the CTR, is a potent inhibitor of bone resorption (1). Accordingly, pharmacological doses of CT have been used to treat Paget's disease and hypercalcemia, whilst the physiological role of endogenous CT remains unclear (2). Our recent findings point to a complex physiological role of the CTR to inhibit bone formation and to protect against induced hypercalcaemia (1,2). Our aim was to investigate the mechanism by which the CTR exerts these effects by comparing the bone phenotype and hypercalcemic response of global-CTRKOs with a mouse line in which the CTR is deleted specifically in osteoclasts (OCL-CTRKOs). In contrast to global-CTRKOs (1), bone formation was not increased in OCL-CTRKOs at any age. Global-CTRKO and OCL-CTRKOs at 6 weeks of age were fed a low calcium diet for 2 weeks after which hypercalcaemia was induced by treatment with 0.5ug 1,25-dihydroxyvitamin D3 (1,25D). Total serum calcium levels were measured at baseline and 50.5 hours post 1,25D treatment. Consistent with our findings in global-CTRKOs (2), peak serum calcium levels at 50.5 hours post 1,25D-induced hypercalcaemia were greater in OCL-CTRKOs than controls by 20% ($P<0.05$) in males and 18% ($P<0.05$) in females. The elevated hypercalcemic response in global-CTRKOs and OCL-CTRKOs was attributed, at least in part, to increased bone resorption evidenced by increased osteoclast surface ($P<0.05$) and serum X-Laps ($P<0.05$). RANKL:OPG mRNA expression was increased in global-CTRKOs ($P<0.05$) supporting increased osteoclast formation. Tubular reabsorption of calcium was increased in global-CTRKOs ($P<0.01$), suggesting that the protective effect of the CTR against hypercalcemia is also mediated via the kidney. In conclusion, we have shown that the inhibitory actions of the CTR on bone formation are not mediated via the CTR on osteoclasts. Furthermore, the action of the CTR to protect against induced hypercalcaemia is mediated predominantly via its actions on osteoclasts.

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(2) Davey RA et al, 2008 JBMR 23:1182-93

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EFFECTS OF PARATHYROID HORMONE DEFICIENCY AND EXCESS ON CORTICAL AND TRABECULAR MICRO-ARCHITECTURE

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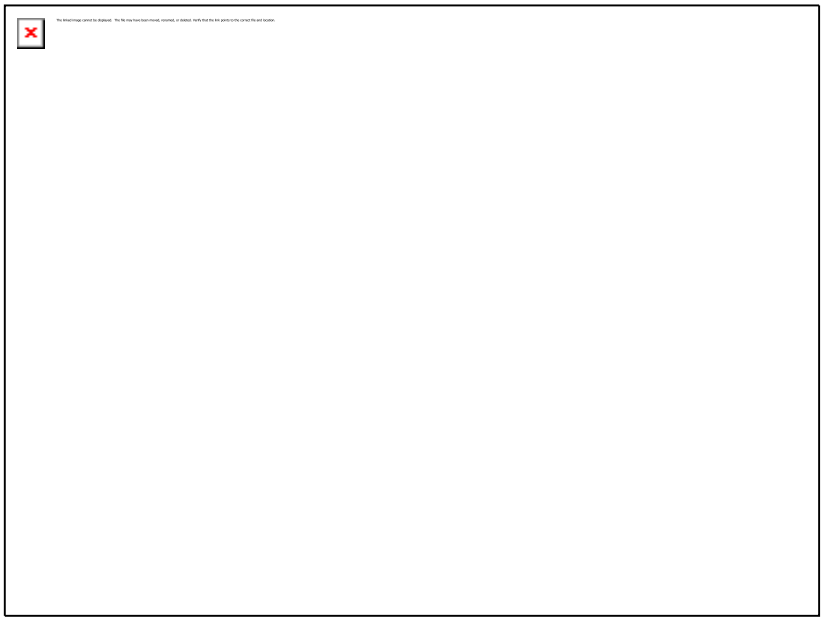
Parathyroid hormone (PTH) excess is believed to produce cortical thinning by endocortical resorption but may be anabolic at trabecular sites. [1] However, increased intracortical remodelling induced by PTH produces coalescence of pores in cortex adjacent to marrow 'trabecularizing' it. This falsely elevates trabecular density while thinning the cortex from within. [2] We examined the effects of PTH deficiency and excess on cortical and trabecular micro-architecture using high resolution peripheral quantitative computed tomography in 10 patients with hypoparathyroidism (HypoP), mean age 61 + 11 years; 13 patients with untreated primary hyperparathyroidism (HyperP), mean age 63 + 14 years, and 10 patients with treated HyperP, mean age 61 + 15 years. Morphology was expressed as z scores (mean ± sem).

Hypoparathyroid patients had a non-significant increase in distal radial total volumetric bone mineral density (vBMD, 0.43+0.24) in addition to increased cortical parameters: area (0.68+0.35, $p>0.05$), density (0.35+0.31, $p>0.05$) and thickness (0.53+0.2, $p<0.05$) with normal trabecular parameters. Untreated HyperP patients had decreased total vBMD (-0.29+0.26, $p>0.05$) as well as cortical area (-0.61+0.35, $p>0.05$) and density (-0.78+0.39, $p<0.05$) with normal cortical thickness (-0.11+0.10, $p>0.05$) and trabecular parameters. Treated HyperP had non-significant reductions in total vBMD (-0.51+0.32), cortical area (-0.50+0.32), density (-0.30+0.42) and thickness (-0.23+0.18) and also reduced trabecular density (-0.66+0.25, $p<0.05$) due to reduced trabecular number (-0.79+0.31, $p < 0.05$), increased trabecular separation (0.77+0.38, $p < 0.05$) but normal area (-0.07+0.29) and thickness (-0.02+0.32). Similar data were found at the tibia (not shown).

While inferences are constrained by the small sample size in this pilot study, the data suggest that PTH excess is deleterious to cortical bone reversibly increasing intracortical porosity and artifactually increasing trabecular density. After treatment, the irreversible deficit in trabecular bone produced by loss of trabeculae number (not thickness) appear as cortical deficits are reversed.



(1) Seeman et al. Journal of Clinical Investigation 1982; 69(6): 1302 - 1309
(2) Zebaze et al. Abstract 1101 ASBMR 2008

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NEUROENDOCRINE REGULATION OF GROWTH HORMONE AND ANDROGEN STATUS BY SELECTIVE OESTROGEN RECEPTOR MODULATORS IN HEALTHY MEN

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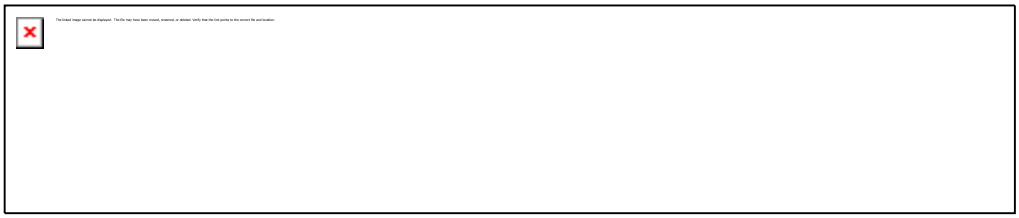
In men, the stimulation of GH and inhibition of LH secretion by testosterone (T) requires aromatisation to oestradiol. Tamoxifen and raloxifene are Selective Oestrogen Receptor Modulators (SERMs) that block central oestrogen action. The aim was to investigate whether SERMs in therapeutic doses attenuate the GH-IGF-I and stimulate the gonadal axes in healthy men.
Ten healthy men were randomised to 2-week sequential treatment with tamoxifen (Tam; 10 and 20 mg/d) and raloxifene (Ral; 60 and 120 mg/d) with washout of 2 weeks in between. Peak GH response to arginine stimulation, serum IGF-I, LH, T, and SHBG levels were measured.
Neither tamoxifen nor raloxifene significantly affected the peak GH response to arginine. Tamoxifen but not raloxifene reduced IGF-I levels and increased SHBG levels. Both drugs increased LH and testosterone concentrations. The increase in testosterone was greater with tamoxifen than with raloxifene treatment.



* $p<0.05$ vs baseline; # $p<0.05$ vs Ral 120mg

In summary, tamoxifen but not raloxifene suppress the GH-IGF-I axis. Both tamoxifen and raloxifene stimulate the gonadal axis with tamoxifen imparting a greater effect. In conclusion, in therapeutic doses, tamoxifen perturb central regulation of the GH and gonadal axes to a greater degree than raloxifene.

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THE ROLE OF UNILATERAL ADRENALECTOMY IN BILATERAL PRIMARY ALDOSTERONISM - A 22 YEAR SINGLE CENTRE EXPERIENCE

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Context: Primary aldosteronism (PA) due to bilateral autonomous aldosterone production is usually treated medically. Unilateral adrenalectomy (U-ADX) has been considered ineffective. Because quality outcome data are lacking, and medical treatment may cause adverse effects or fail to control hypertension, defining the role for U-ADX in bilateral PA is an important clinical issue.

Design and Setting: We examined BP, biochemical and echocardiographic responses to U-ADX in patients with bilateral PA and sought predictive parameters. Between 1984-2004, 51 of 684 patients diagnosed with bilateral PA based on adrenal venous sampling (ipsilateral aldosterone/cortisol ratio ≥twice peripheral, contralateral suppression) underwent U-ADX. This report is based on the 40 followed for ≥12 (median 56.4) months, lacking other secondary endocrine or renal causes of hypertension, and not treated with aldosterone antagonists post-operatively. Reasons for surgery included intolerance of, or inadequate BP response to aldosterone antagonists (22), strong patient preference (9) or an adrenal mass warranting removal based on malignant potential (9).

Results: Hypertension was cured (≤140/90, no drugs) in 15% and improved (fewer drugs, dosage not increased; or dosage decreased, no added drugs) in 20% , usually within one year of surgery. The proportion with BP ≤140/90 was significantly ($p<0.001$) higher after U-ADX (65%) than before (25%). There were significant falls in mean systolic BP (from 151±4 SEM at presentation to 138±3 mmHg; $P<0.01$), diastolic BP (94±2 to 84±1 mmHg; $P<0.001$), left ventricular mass index (106±4 to 95±3 g/m2; $P<0.05$), plasma upright aldosterone (643±55 to 527±72 pmol/L; $P<0.05$) and aldosterone/renin ratio (301±44 to 109±23; $P<0.001$). Lower serum creatinine independently predicted hypertension cure.

Conclusion: Although this retrospective analysis of patients from a single center does not permit prediction of response rates among patients diagnosed elsewhere, it suggests that U-ADX can be beneficial in some patients with apparently bilateral PA and should not be dismissed as a treatment option.

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A longitudinal study on the physiological changes in total plasma cortisol and 24-h urinary free cortisol levels during pregnancy and post-partum, compared with non-pregnant subjects and subjects on low dose oral contraceptive pill

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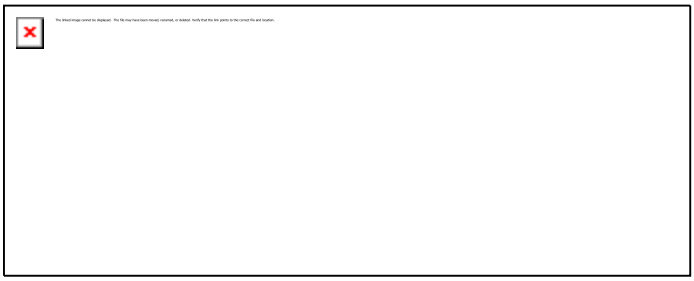
Previous studies reported total plasma cortisol (PC) and 24-h urinary free cortisol (UFC) levels increase 2-3 fold during pregnancy based primarily on cross-sectional data. There is a paucity of longitudinal pregnancy data using modern immunoassays. Furthermore, conflicting data exists as to the effect of exogenous oestrogen, with some studies reporting that combined oral contraceptive pill (OCP) containing low dose oestrogen (≤35mcg ethinyloestradiol) has no effect on PC. We conducted a prospective longitudinal study on PC (measured between 8-9am) and 24-h UFC levels in 14 pregnant women during the first (T1, between 8-14 weeks), second (T2, 18-24 weeks), third (T3, 30-36 weeks) trimesters and 2-3 months post-partum, compared to cortisol levels in 10 non-pregnant subjects not on an OCP (control group) and 9 subjects taking the low dose OCP. The results (mean ± SEM) are shown in the table. A progressive rise in PC was noted during pregnancy (mean 2-fold rise compared to controls, peaking during T2 and T3), and a similar increase in PC was seen in the OCP group. The mean UFC levels increased 3-fold by T2 compared to controls, and increased further during T3, whereas the mean UFC in the OCP group was not statistically different to controls. Our study demonstrated elevations in PC levels during pregnancy and with low dose OCP use, presumably due to the oestrogen-stimulated increase in corticosteroid-binding globulin levels. Importantly, pregnancy was also associated with significant increases in UFC levels during T2 and T3, suggesting that other physiological changes occur in the regulation of the maternal hypothalamic-pituitary-adrenal axis. Given our findings, we recommend that pregnancy-specific reference ranges for PC and UFC need to consider gestational age at time of testing, and the interpretation of PC levels in non-pregnant women need to consider whether patients are taking exogenous oestrogens.



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EVIDENCE FOR CENTRAL REGULATION BY ESTROGEN OF GH SECRETION IN WOMEN: A STUDY OF THE EFFECTS OF ESTROGEN RECEPTOR ANTAGONISM

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In men, stimulation of GH secretion by testosterone requires prior aromatisation to estrogens, an effect unmasked by estrogen receptor blockade with tamoxifen. Estrogen supplementation by a physiological non-oral route fails to stimulate GH secretion in postmenopausal women. While this observation has questioned the role of circulating estrogen, it does not exclude an effect of estrogens produced locally from aromatisation.

To investigate a putative role of estrogen on the GH system, we studied the effects of tamoxifen in ten healthy postmenopausal women (age 64±2 years) on the peak GH response to arginine, and on IGF-I and SHBG levels. Measurements were obtained before and after treatment with 10 and 20 mg/d tamoxifen for two weeks each in an open-label sequential study.

When compared to baseline, peak GH concentration was reduced significantly by the 20 mg dose (13.6±4.4 vs 4.2±1.4 mIU/L; $p<0.05$) but not the 10 mg dose of tamoxifen (9.4±2.8 mIU/L). Mean IGF-I concentration was also reduced significantly by the higher (16±1.6 vs 12.3±1.6 nM/L; $p<0.01$) but not the lower dose of tamoxifen (14.5±1.6 nM/L). Mean SHBG levels increased significantly ($p<0.01$) in a dose dependent manner from 48.3±7.9 to 60±9.4 and 61.9±9.9 nM/L, respectively.

In summary, in postmenopausal women, tamoxifen reduced peak GH response to arginine and decreased IGF-I but increased SHBG levels. The increase in SHBG is consistent with a known hepatic estrogen agonistic effect which is likely to inhibit IGF-I production from the liver. The finding of a blunted peak GH response to stimulation despite reduced IGF-I feedback inhibition indicates profound central suppression of GH output by tamoxifen. This study demonstrates that estrogens regulate central GH secretion in women. The contrasting effects on GH status between estrogen supplementation and receptor blockade suggest a paracrine mechanism and an important role of aromatase in the neuroregulation of the GH system.

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ROLE OF THE CELL SURFACE ESTROGEN RECEPTOR, GPR-30 IN CONTRACTILITY OF HUMAN PREGNANT MYOMETRIUM

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Context: Traditionally, estrogen binds to the nuclear receptors, ER-α and ER-β. Recently a novel cell surface receptor for estrogen, called GPR30, was identified in cancer cells. Objective: We investigated the presence of GPR30 in myometrium from pregnant women at term and the non-genomic signalling pathways activated by this receptor. Methods: Myometrial tissues were collected from elective or emergency caesarean sections. Tissues were immediately frozen in liquid nitrogen and stored at -80° C. These tissues were used for RNA and protein extraction. We performed realtime quantitative PCR and western blots for GPR30 detection. We also performed fresh human myometrial tissue culture to examine the phosphorylation of MAPK (p42/44) and heat shock protein 27 (hsp27) and expression of connexin-43 in response to G1, a GPR30 specific agonist. We also suspended myometrial tissue strips in organ bath after 12 hrs treatment of estradiol and G1. Strips were then connected to a Grass FT03C force transducer. Contraction force of muscle strips was continually measured after addition of different doses of oxytocin. Results: mRNA for GPR30 was detected in pregnant myometrium at term. Western blot analysis revealed that a monomeric ~38 kDa protein band specific for GPR30 was expressed in human pregnant myometrium at term. In addition, a trimeric form was also detected at ~120 kDa. In myometrial tissue culture, treatment with the GPR30 specific agonist G1 induced phosphorylation of p42/44 MAPK as well as hsp27 within 45 mins. Interestingly, G1 also can increases the expression of connexin-43 in a dose dependent manner within 4 hrs in myometrial tissue culture. Additionally, G1 can augment contractility of myometrium and responsiveness to oxytocin. Conclusion: This study documents the novel detection of GPR30 in human myometrium. Preliminary investigations reveal a potential role for this receptor in regulating the contractile machinery of the myocytes, by phosphorylating hsp-27 and p42/44 and by increasing the expression of connexin-43.

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ESTROGEN RECEPTOR A FORMS STRONG INTER-RELATIONSHIPS WITH EXPRESSION OF INFLAMMATORY, CONTRACTION-ASSOCIATED AND NOVEL GENES IN THE HUMAN MYOMETRIUM BEFORE AND DURING LABOUR

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Context: Estrogens increase at the time of human labour. The main uterine receptor ERα is also up-regulated in labour, however, the genes activated by this nuclear receptor are unknown.

Objective: To examine the relationships between the expression of ERα and established and novel parturition-related genes in human myometrium.

Design: Quantitative expression of two panels: known and novel parturition-associated genes in myometrial tissues from women either not in labour (NL) or in labour (L).

Setting: John Hunter Hospital, NSW, Australia, and KK Women's and Children's Hospital, Singapore, both tertiary care obstetric institutions.

Patients: Pregnant women who had elective (n=37) or emergency (n=29) Caesarean sections at ≥ 37 weeks gestation.

Main Outcome Measures: Quantitative RTPCR of relative mRNA abundances of 25 genes.

Results: Expression of known contraction-associated genes (CX43, COX2, PTGFR, PTGER1-2) is significantly increased during labour but not the oxytocin receptor. ERα is strongly positively correlated with CX43 and COX2 both prior to and during labour. Eight genes (CRSP6, IFITM2, KIAA, NAPG, NPM1, PGCP, SERPINF1 and TPI1) not previously linked to human parturition are up-regulated in the myometrium in labour. Multiple linear regression shows that CX43, PGCP, KIAA, SERPINF1 and H2AFZ are simultaneously and significantly associated with ERα expression. Structural Equation Modelling shows that ERα and inflammatory gene changes precede changes in contraction-associated proteins and most of the variance in expression of critical genes can be accounted for by our modelling. Conclusion: These data support critical roles for ERα and inflammatory genes in coordinating myometrial gene activation at the time of human labour.

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HYPOTHALAMIC EXPRESSION OF THE KISSPEPTIN GENE (KISS1) AND THE RFAMIDE-RELATED PEPTIDE (RFRP) GENE DURING THE MENSTRUAL CYCLE OF A NON-HUMAN PRIMATE

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Kisspeptin is the product of the *Kiss1* gene that binds to the receptor GPR54 and stimulates gonadotrophin-releasing hormone (GnRH) secretion. RFamide-related peptide (RFRP-3) is encoded by the *RFRP* gene and inhibits the reproductive axis. In sheep, Kiss1 expressing cells are found in the arcuate nucleus (ARC) and preoptic area (POA) and mediate the feedback effect of estradiol to generate the preovulatory GnRH/LH surge. The RFRP gene is localised in cells of the ovine paraventricular nucleus (PVN) and dorsomedial hypothalamic nucleus (DMN). In the primate, Kiss1 cells are found in the ARC, but whether they regulate GnRH cells is unknown. A population of Kiss1 cells in the POA, or whether RFRP cells are found in the primate brain, and what function the encoded neuropeptides play in this species is also unknown. We examined the expression of Kiss1 and RFRP mRNA throughout the ovarian cycle of the female monkey (*Macaca mulatta*), using *in situ* hybridization. Kiss1 expressing cells were found in the POA and ARC, and RFRP expressing cells were located in the PVN-DMN region of the primate brain. Kiss1 mRNA expression in the caudal ARC and the POA was higher ($P<0.05$) in the late follicular phase of the cycle (just prior to the GnRH/LH surge) (n=4) than in the luteal phase (n=3). RFRP gene expression was unchanged during the ovarian cycle. We ascertained whether kisspeptin and/or RFRP cells project to GnRH neurons in the primate. Close appositions of kisspeptin immunoreactive varicose fibres were found on 17% of GnRH neurons within the mediobasal-hypothalamus and RFRP-3 appositions were found on 20% of GnRH neurons; both were similar across the ovarian cycle. These data suggest kisspeptin neurons are involved as central processors for the preovulatory GnRH/LH surge in the non-human primate. The role RFRP neurons play in the primate is yet to be determined.

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GLUCOCORTICOID RECEPTOR CHAPERONE MOLECULES AND SEX-SPECIFIC SENSITIVITY TO CORTISOL IN THE HUMAN PLACENTA

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Fetal growth inhibition is a known sequelae of in-utero glucocorticoid exposure. In pregnancies complicated by asthma, elevations in maternal cortisol are associated with reduced growth trajectories in female but not male fetuses. Cortisol exerts its biological effect through binding with the glucocorticoid receptor (GR). In its unbound form, this receptor resides in the cytoplasm coupled with a chaperone complex, including heat shock proteins 70 and 90 (HSP70 and HSP90) that coordinate protein movement, folding and activation. The present study aimed to investigate whether placental glucocorticoid sensitivity differed in a sexually dimorphic manner in pregnancies complicated by asthma. Further, expression of HSP70 and HSP90 were measured as a potential mechanism for altered sensitivity. Following term delivery, placentae were collected from control (n=13) and asthmatic women (n=26) and infant sex was recorded. Placental explants were incubated with LPS (1ng) with and without cortisol for 2 hours, and supernatant concentrations of pro-inflammatory cytokines (TNFα and IL-1β) were measured by ELISA. Placental RNA was extracted and RT-PCR was used to measure HSP70 and HSP90, and GRα protein was determined by Western Blot. Placental GRα protein was equally expressed in males and females of the asthmatic and control groups. However, cortisol significantly inhibited placental TNFα production only in placentae from asthmatic women ($p<0.01$) regardless of fetal sex. Expression of HSP70 and HSP90 were significantly lower in male compared to female placentae in the asthmatic group ($p<0.03$ in each instance), but did not differ between the sexes within the asthmatic or control groups. These data suggest that while no difference in GRα protein levels occur with asthma between the sexes, differential sensitivity to cortisol may be due to decreased expression of co-chaperone molecules that alter functional properties of placental GRα.

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THE EFFECT OF ENDURANCE EXERCISE WITHOUT ENERGY RESTRICTION ON INSULIN RESISTANCE MEASURED BY EUGLYCAEMIC HYPERINSULINAEMIC CLAMP AND BODY COMPOSITION IN OBESE WOMEN WITH AND WITHOUT POLYCYSTIC OVARY SYNDROME

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Polycystic Ovary Syndrome (PCOS) is an insulin resistant (IR) state. Exercise without energy restriction (which may be more sustainable than dietary interventions) improves reproductive parameters in PCOS and fasting insulin, an insensitive marker of IR (1) . However mechanisms by which exercise improves IR in PCOS have not been elucidated. Visceral fat(VF) correlates strongly with IR in PCOS, however the reduction of IR with metformin doesn't reduce VF (2) . VF reduction may occur without significant weight loss with exercise in obesity (3) . More IR offspring of diabetics show better response to exercise than less IR controls (4) . Computer tomography (CT) accurately delineates visceral *vs.* abdominal subcutaneous fat and has not been studied in this setting. This study compares the effect of exercise without energy restriction on body composition and IR in overweight women with PCOS compared to weight matched non-PCOS women.

Design: Glucose infusion rate (GIR) on euglycemic hyperinsulinemic clamp, fat distribution by CT, dual-energy x-ray absorptiometry (DEXA), maximal aerobic capacity (VO_{2max}) were assessed in 17 overweight PCOS women (BMI >27 kg/m2) and 12 BMI-matched controls at baseline, and 13 PCOS and 8 controls after 12 weeks of intensified endurance training (1 hour 3 times/week).

Results: At baseline, PCOS were more IR than controls and IR correlated with VF. With exercise, VO_{2max} improved similarly in both groups. There was no significant weight loss in either group. In PCOS, IR improved and VF on CT decreased significantly (p=0.03) (consistent with decreased android fat on DEXA). In contrast, abdominal subcutaneous fat (p=0.01) and waist circumference decreased in the non-PCOS women.

Conclusions: This study suggests that improvement in IR with exercise may be related to a decrease in VF in obese women with PCOS. Mobilisation of VF may be more highly modulated by physical activity in women with PCOS than controls.

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(3) Kay SJ, Singh MAF: The influence of physical activity on abdominal fat: a systematic review of the literature. Obesity reviews 7:183-200, 2006
(4) Barwell ND et al. Exercise training has greater effects on insulin sensitivity in daughters of patients with type 2 diabetes than in women with no family history of diabetes. Diabetologia 51:1912-1919

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THE USE OF ACTH IN ADRENAL VENOUS SAMPLING IMPROVES LATERALIZATION OF ALDOSTERONE OVERSECRETION IN PRIMARY ALDOSTERONISM PATIENTS

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Background: Adrenal venous sampling (AVS) remains the gold standard in lateralization in primary aldosteronism [1]. ACTH stimulation was initially proposed to reduce aldosterone and cortisol fluctuations during sampling, however its usefulness remains controversial [2]. The aim of this study is to assess a) the pulsatility of aldosterone and cortisol post ACTH administration, b) whether ACTH adds value to the interpretation of AVS results.

Methods: 41 patients had AVS performed with paired pre and post ACTH results during 2000-2007. Cortisol and aldosterone samples were collected sequentially from the adrenal veins (AV) and peripheral vein (PV) in duplicates pre and post ACTH. Lateralization required: a) successful cannulation of adrenal veins, b) aldosterone cortisol ratio (ACR) of the dominant side $\geq$ 4 times of the non-dominant side, and c) non-dominant side $_{ACR}$ < PV_{ACR}. Results were inconclusive if cannulation was unsuccessful, or if non-dominant $_{ACR}$ failed to suppress to PV_{ACR} when dominant$_{ACR} \geq$ 4 times non-dominant $_{ACR}$.

Results: Post ACTH stimulation, the coefficient of variation of aldosterone was reduced by 59% (p = 0.014), the rate of successful cannulation was increased by 28% (p=0.04), and the number of conclusive AVS interpretation increased by 56% (p=0.003). Of the 20 patients with inconclusive pre ACTH results, 8 had no lateralization post ACTH and avoided adrenalectomy, 5 had lateralization post ACTH and 1 proceeded to adrenalectomy with resolution of hypokalemia and hypertension. For the total 17 patients who proceeded with adrenalectomy, ACTH stimulation provided additional information in 12% of patients.

Conclusion: The use of ACTH during sequential AVS reduced aldosterone pulsatility, improved cannulation rate and potentially altered management in 12% of patients who proceeded to surgery. The use of ACTH should be considered in centres without dedicated AVS radiologists to improve the cannulation rate and the yield of this invasive procedure.

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BROWN ADIPOSE TISSUE IN HUMANS: PREVALENCE, ANTHROPOMETRIC AND METABOLIC PREDICTORS

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Brown adipose tissue (BAT) plays a major role in energy homeostasis in animals. The view that BAT disappears after infancy in humans has been challenged by the detection with PET-CT of adipose tissue with high metabolic activity. AIM: To determine the prevalence of BAT in humans. METHODS: A retrospective evaluation of consecutive PET-CT scans, using 18F-fluodeoxyglucose (18F-FDG), in oncology patients at the Royal Prince Alfred Hospital between 2003-2008 was undertaken to determine the presence of putative BAT. BAT was identified as areas of avid FDG uptake within fat delineated by CT. Age, sex, disease activity, body mass index (BMI) and fasting blood glucose concentration were assessed for association with presence of BAT. RESULTS: A total of 4834 consecutive PET-CT scans performed on 2934 patients were analysed. Positive scans were identified in 250 patients, yielding a prevalence of 8.5%. Among 747 patients who were scanned more than once, at least one positive scan was identified in 145 patients, yielding a much higher prevalence of 19.4%. BAT was detected most commonly along the cervico-thoracic area. Patients with BAT were significantly younger (36±1 vs 52±1 years, $p<0.001$) with lower BMI (20.1±0.1 vs 24.9±0.2 kg/m2, $p<0.001$) and fasting blood glucose concentration (4.7±0.2 vs 5.5±0.3 mmol/L, $p=0.01$) than those with no BAT. In the group of patients who were scanned more than once (mean scanning interval: 7.2±1.1 months), body weight and fasting glucose were significantly lower (54.9±0.5 vs 58.2±0.8 kg, $p<0.001$ and 4.9±0.3 vs 5.5±0.3 mmol/L, $p=0.03$, respectively) on occasions when BAT was detected compared to when BAT was absent. SUMMARY: BAT is present in up to 1 in 5 patients and correlate negatively with BMI and glycaemia. CONCLUSION: BAT plays a regulatory role in energy metabolism in adult humans and may be a future target of obesity treatment. (supported by NHMRC Australia)

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BONE ARCHITECTURAL DECAY IN MALES WITH PROSTATE CANCER TREATED WITH ANDROGEN DEPRIVATION THERAPY

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Introduction: Sex steroids influence bone remodelling. Androgen deprivation therapy (ADT) for treatment of prostate cancer reduces bone mineral density (BMD), but the structural basis of the BMD deficit has not been determined prospectively. The new technique, high resolution peripheral quantitative CT (HR-pQCT) detects changes in bone microarchitecture. However, increased intracortical remodeling leaves pores in cortex adjacent to marrow, which coalesce producing cortical remnants which look like trabeculae (trabecularization) and may be erroneously included in the trabecular density measurement.

Aim: We hypothesized that ADT will reduce cortical and trabecular thickness, increase cortical porosity and reduce serum testosterone levels in males treated with ADT for non-metastatic prostate cancer.

Methods: 21 males, mean age 71 years, with non-metastatic prostate cancer were studied at baseline, 6 and 12 months after commencing ADT. Assessment comprised medical history, physical examination and fasting blood analyses including sex-hormones. BMD was determined by dual energy x-ray absorptiometry (DEXA) and microarchitecture was assessed using HR-pQCT.

Results: ADT decreased total testosterone levels (12.8 to 0.8nmol/L, $p<0.001$). Microarchitecture changed at the distal tibia with a decrease in average bone density (-4.0%), due to a decrease in cortical density (a surrogate of porosity increase) (-3.6%) and cortical thickness (-10.2%, all $p<0.001$) not trabecular density. At the radius there was a decrease in average bone density (-6.2%) due to a decline in cortical density (-3.2%) and cortical thickness (-11.5%, all $p<0.001$) as well as trabecular density (-3.2%, $p=0.028$). At both the tibia and radius, the cortical area decreased and trabecular area increased (all $p< 0.001$). BMD decreased at the lumbar spine (-3.6%, $p<0.001$) and total femoral neck (-2.6%, $p=0.001$).

Conclusions: Testosterone deficiency induced by ADT for prostate cancer, results in decay of cortical and trabecular bone. The decrease in cortical bone and increase in trabecular area may be due to trabecularization of cortical bone.

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THE FETAL COTYLEDON IS A MAJOR SOURCE OF MATERNAL CIRCULATING C-TYPE NATRIURETIC PEPTIDE IN OVINE PREGNANCY

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C-type natriuretic peptide (CNP) is a paracrine growth factor that circulates in healthy adults at barely detectable concentrations (< 1 pmol/l). However in ovine pregnancy, maternal CNP is substantially elevated, increasing at the end of the first trimester to achieve sustained concentrations (> 20 pmol/l) until values decline rapidly within the last week of gestation. These findings suggest major contributions from the gravid uterus to circulating maternal CNP concentration [1], but relative contributions from uterine and placental tissues are unknown. Accordingly we have measured concentrations of CNP and the amino terminal fragment of proCNP [2] (NTproCNP) in uterine and placental tissue (separating maternal caruncle from fetal cotyledon) at five stages of gestation (days 30, 60, 100, 135 and at term) and compared the values with time matched maternal plasma CNP concentration in 20 pregnant ewes. Concentrations of CNP forms in placental tissues greatly exceeded those in uterine tissues at all time periods ($p < 0.05$). Caruncular concentrations (CNP 32 ± 4 pmol/g, NTproCNP 56 ± 6 pmol/g) peaked at day 60 whereas in the cotyledon there was a progressive increase in both CNP forms to peak values (CNP 66 ± 6 pmol/g, NTproCNP 134 ± 9 pmol/g) at day 100-135. In the cotyledon, concentrations of both forms had declined sharply at day 143. Changes in maternal plasma concentration of both CNP and NTproCNP closely followed those in the cotyledon. Size exclusion HPLC analysis of cotyledon extracts confirmed that the immunoreactive material was the same as the CNP forms previously identified in the maternal circulation [1]. These findings indicate that CNP synthesis by fetal cotyledons is a major source of maternal hormone concentrations in ovine pregnancy. Because maternal circulating CNP forms reflect placental production, the pregnant ewe may provide an unique model for study of placental CNP regulation in vivo.

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ROLE OF SUPPRESSOR OF CYTOKINE SIGNALLING-3 (SOCS-3) IN THE NEUROENDOCRINE REGULATION OF FERTILITY IN MICE FED A HIGH FAT DIET

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Fertility in the western world has been decreasing (1), and at the same time the average age of puberty has been decreasing (2), both of these have been correlated with an increased consumption of high caloric foods leading to an increase in the incidence of obesity (3, 4). A critical neuroendocrine signal for puberty onset and fertility is the hormone leptin, which is produced by adipose tissue. Obese individuals possess more adipose tissue and as a result have elevated leptin levels. In the long term this can induce leptin resistance by mechanisms such as elevated levels of SOCS-3 in the hypothalamus. This experiment tests whether SOCS-3 plays a role in fertility suppression in animals fed a high fat diet (HFD). Using CRE/LoxP transgenics, we selectively knocked out SOCS-3 from all forebrain neurons in both male and female DBA/2J mice. Neuronal SOCS-3 knockout mice and control littermates were fed either a HFD (23%) or standard chow diet from weaning and subjected to a comprehensive fertility analysis. Onset of puberty (measured as the date of first oestrus in females or birth of first successful mating in males) was advanced by 4.9 days in male but not female wild-type mice fed a HFD when compared to chow-fed littermates ($P<0.01$). In addition, the body weight at puberty onset of neuronal knockout mice fed a HFD was on average 2.2 g less than that of wild type littermates ($P<0.05$ for both sexes). These results show that HFD reduces the age of puberty onset in DBA/2J mice. Furthermore, increased leptin signalling due to an absence of inhibition by SOCS-3 can induce puberty at a lower body weight than wild type littermates. Long term adult fertility data is currently being assessed in these mice.

Supported by the Health Research Council of New Zealand

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STAT5 IS ACTIVATED BY LEPTIN IN THE HYPOTHALAMUS BUT IS NOT REQUIRED FOR REGULATION OF FERTILITY

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Leptin signalling in the hypothalamus is mostly attributed to signal transducer and activator of transcription 3 (STAT3), but it has recently been suggested that signalling through distinct pathways such as STAT5 may mediate leptin's effects on fertility (1). However a role for STAT5 in leptin-regulated neuroendocrine processes is unknown. To test if leptin can induce phosphorylation of STAT5 in the hypothalamus, mice were administered a single injection of leptin (1 mg/kg i.p.) or vehicle 60 minutes prior to perfusion. Immunohistochemistry was carried out to determine the number of phosphorylated STAT5 (pSTAT5) immunoreactive cells in the arcuate nucleus (ARC). Leptin markedly induced pSTAT5 in the ARC (vehicle-treated, 20 ± 5; leptin-treated, 155 ± 12 cells/section; $p<0.001$). To test if STAT5 signalling in the brain is required for fertility, STAT5 was conditionally knocked out of forebrain neurons in mice using Cre-LoxP transgenics and male and female fertility assessed. There was no difference in puberty onset between control and neuronal STAT5 knockout mice (female vaginal opening and first estrous in the two groups averaged 28.5 ± 2.6 and 31.7 ± 2.4 days respectively; male first successful mating averaged 41.3 ± 1.0 days; all $P>0.05$). Estrous cyclicity (assessed over 10 days by vaginal cytology) was normal in both controls and knockouts. Experimental mice were mated with wild-type mice to determine adult fertility. There was no effect of neuronal STAT5 knockout on the number of litters produced (males averaged 3.3 ± 0.2 litters/100days; females averaged 3.5 ± 0.2 litters/100days; both $P>0.05$) or litter size (males averaged 7.8 ± 0.3 pups/litter; females averaged 8.6 ± 0.5 pups per litter; both $P>0.05$). These data confirm that leptin can activate STAT5 in the hypothalamus; however they also show that hypothalamic STAT5 is not required for fertility. Since leptin-induced STAT3 signalling does not appear to be critical for fertility either, it may be that leptin is able to act through either pathway in a redundant fashion.

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THE SYNTHETIC PROGESTIN MEDROXYPROGESTERONE ACETATE INCREASES HUMAN BREAST EPITHELIAL CELL PROLIFERATION BY DISRUPTING ANDROGEN RECEPTOR SIGNALLING

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Postmenopausal women using combined (oestrogen and progestin) hormone replacement therapy (CHRT) containing the synthetic progestin medroxyprogesterone acetate (MPA) have an increased risk of breast cancer. MPA binds the progesterone receptor (PR) and the androgen receptor (AR) with high affinity and can thereby stimulate more complex molecular events than natural progesterone. AR signalling inhibits breast epithelial cell proliferation and we propose the hypothesis that MPA increases breast cancer risk by interacting with the AR and disrupting androgen-stimulated AR signalling, leading to loss of normal growth homeostasis. Histologically normal breast tissue was collected from 12 post-menopausal women following surgery. A piece of tissue was processed for immunohistochemical analysis of AR and PR. The remainder was cut into 3 mm^3 pieces and cultured in triplicate on gelatine sponges soaked in steroid deplete media containing the following treatments: vehicle control (EtOH), 5α-dihydrotestosterone (DHT;1nM), MPA(1nM) and MPA+DHT. After 24 hours, tissues were processed for immunohistochemical analysis of AR, a proliferation marker (Ki67), and a pro-survival factor (bcl-2). Breast tissues had a median 7-fold higher level of AR compared to PR ($p<0.00001$). Treatment with DHT and MPA alone significantly increased AR immunoreactivity to an equal degree ($p=0.04$ for both treatments) compared to control. DHT tended to decrease the number of Ki67 positive cells ($p=0.08$) but MPA had no effect ($p=0.48$). Importantly, the combination of MPA+DHT resulted in significantly increased Ki67 positivity ($p<0.0001$ and decreased AR immunoreactivity ($p=0.03$) compared to DHT treatment alone. Hormone treatments did not significantly alter bcl-2 immunoreactivity. In conclusion, AR is more abundant than PR in post-menopausal breast tissue and MPA can interact with AR and inhibit DHT-stimulated effects that result in an overall increase in breast epithelial cell proliferation. This action of MPA could feasibly contribute to the increased risk of breast cancer in women who use CHRT.

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INCREASED ADIPOSITY IN ANDROGEN RECEPTOR KNOCKOUT MICE

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We previously showed our global androgen receptor knockout (ARKO) male mice have increased subcutaneous and perirenal fat mass compared to wildtype (WT) males, with decreased voluntary activity. Microarray analysis on subcutaneous fat from WT and global ARKOs has identified >4000 differentially expressed genes, including leptin, adiponectin and lipoprotein lipase, which is currently being confirmed by real-time PCR. To determine if the increased adiposity in global ARKOs is due to androgen actions in skeletal muscle, we examined muscle-specific ARKO (mARKO) males generated using MCK-cre (expressed in myotubes). Expression of the AR gene is decreased by 95% in mARKO muscle versus WT ($p<0.001$, n=12/group). Subcutaneous fat mass is 22% higher in mARKOs versus WT ($p<0.001$, n=20/group); however, is also 20% higher in AR^{lox} males ($p<0.05$, n=30/group), which have the floxed AR gene but no cre

transgene, and there is no significant difference between mARKO and AR^{lox} males. Similarly, perirenal fat mass is 60% higher in mARKOs versus WT ($p<0.001$), and 43% higher in AR^{lox} versus WT ($p<0.05$), with no significant difference between mARKO and AR^{lox} males. Preliminary data show no difference in voluntary activity of mARKO males (n=3/group). We believe the increased adiposity in these mice may be due to the AR^{lox} allele rather than the muscle-specific AR deletion. However, AR gene expression is normal in subcutaneous and perirenal fat from mARKO versus WT (n=12/group); therefore, the cause of the increased adiposity remains undetermined. To determine if androgens play a physiological role in regulating fat mass in females, we are generating global ARKO females. Preliminary data show no difference in fat mass between AR^{lox} heterozygous and WT (n=3-6/group), and we are currently collecting sufficient ARKO females to perform statistical analysis. Our results suggest that increased adiposity in mARKO males is not associated with decreased voluntary activity however, this still requires further investigation.

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RANDOMIZED PLACEBO-CONTROLLED CLINICAL TRIAL OF TRANSDERMAL DIHYDROTESTOSTERONE GEL DOES NOT INFLUENCE PROSTATE GROWTH RATE AND REDUCES SPINAL BUT NOT HIP BONE DENSITY OVER 24 MONTHS

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Whether DHT, a non-amplifiable, non-aromatizable pure androgen, may reduce prostate growth in middle-aged men (1) was evaluated in a randomized, controlled trial of daily transdermal DHT (70 mg daily, Besins)vs matching placebo gel for 2 years in healthy men over 50 year old without known prostate disease. Total and central prostate volume (by ultrasound), bone mineral density (BMD by DEXA), blood reproductive hormones (LH, FSH, SHBG, PSA by immunoassay; steroids by LC tandem MS) and biochemical markers of bone turnover (formation, serum P1NP; resorption, urine NTX) by immunoassay were measured every 6 months. Participants (n=114, 92% Caucasian, median age 59 years) demonstrated high compliance (96%) and completion (71%) rates equally for both treatments. DHT administration produced the expected increased blood levels of DHT, 3a and 3b diols with suppression of blood T, LH & FSH but minimal effects on SHBG or PSA. Total and central prostate volumes as well as serum PSA increased progressively over time on study but without difference between DHT and placebo ($p>0.05$). None of the 3 men (all on DHT) discontinued for rise in PSA (>4 ng/mL) had prostate cancer and one man (placebo group) required TURP for BPH. DHT caused significant (within-subject) reduction by ~2% in spine BMD over 24 months whereas hip BMD was not changed. Serum P1NP was markedly increased in the second year of study in DHT but not placebo treated men whereas urine NTX did not change significantly. There was a small increase in lean and decrease in fat mass (DEXA) with DHT but not placebo treatment. There was no significant change in hand grip (dynamometry) strength. Hemoglobin and creatinine were increased reversibly during DHT but not placebo. DHT caused no no serious adverse effects and the only frequent adverse effect was increased hematocrit (15 men causing 8 discontinuations, all DHT) which resolved on discontinuation. We conclude DHT treatment for 24 months has no effect on prostate growth but decreases spinal but not hip BMD. These findings have important implications for the development of non-steroidal androgens.

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GLUCOCORTICOIDS STIMULATE CATECHOLAMINE INACTIVATION IN THE LIVER AND KIDNEY BY DIRECT RAPID INDUCTION OF THE SULFOTRANSFERASE SULT1D1

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Glucocorticoids play a major role in regulating metabolic homeostasis. For example, during periods of fasting glucocorticoids stimulate, via the glucocorticoid receptor (GR), hepatic gluconeogenesis by activating specific gluconeogenic enzyme target genes. To characterise additional hepatic target genes under glucocorticoid control we undertook a gene microarray study using dexamethasone-treated GR-null mice to identify novel GR-regulated hepatic target genes. Strongly induced previously characterized genes included, phosphoenolpyruvate carboxykinase, Serine dehydratase, Lipin 1, metallothionine and Cdkn1A. Novel genes included Ddit4, Fkbp5, Megf9 and Sult1d1 and were subsequently verified by real time PCR. The sulfotransferase enzyme Sult1d1, a member of a large superfamily of detoxification enzymes, was further investigated because of its role in detoxifying endogenous dopamine-derived compounds, including the adrenal catecholamines. Treatment of primary mouse hepatocytes with dexamethasone for six hrs dramatically increased expression of Sult1d1 while co-treatment with RU486, a specific GR antagonist, blocked induction. Sult1d1 mRNA was also increased by dexamethasone in the whole kidney a major site of synthesis of this sulfotransferase and Sult1d1 expression was localised to the collecting ducts by in situ hybridisation. In mouse IMCD3 collecting duct cells Sult1d1 was rapidly induced by dexamethasone in a dose-dependent manner. Sulfotransferase enzyme activities in vivo were found to be significantly higher in kidney and liver tissue after dexamethasone treatment. Analysis of the Sult1d1 gene promoter by CHIP assay identified a glucocorticoid response element ~39kb upstream from the Sult1d1 transcription start site that was close to a neighbouring glucocorticoid-regulated estrogen sulfotransferase, Sult1e1. In summary, Sultd1 in mice is directly and rapidly induced by glucocorticoids to attenuate elevated catecholamines and their derivatives during the stress response and is potentially co-ordinately regulated with the adjacent Sulte1gene via a common distal GRE.

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SRA AND ITS BINDING PARTNER SLIRP HAVE OPPOSING EFFECTS ON APOPTOSIS

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Abrogation of apoptotic mechanisms is a key step in oncogenesis with regulatory pathways being targets for chemotherapy. Nuclear receptor (NR) signaling influences apoptosis in a tissue- and receptor-specific manner with NR coregulators modulating these pathways further. Steroid Receptor RNA Activator (SRA) is unique among NR coregulators as it can act as an RNA coactivator1. While SRA over expression augments the transcriptional activity of a broad range of NRs, its transgenic expression in mouse breast promotes apoptosis and is protective against ras-induced tumour development. Loss of the SRA-binding protein p68 is associated with reduced p53 activity, a major determinant of apoptotic responsiveness. These data suggest SRA and its binding partners may influence apoptotic sensitivity. We have previously described the isolation of the SRA binding, NR corepressor, SRA Stem Loop Interacting RNA-Binding Protein (SLIRP)2. While transcriptional and chromatin immunoprecipitation assays confirm SLIRP's presence and corepressor activity in the nucleus, the majority of the protein resides in the mitochondria, a critical organelle in apoptosis. As a consequence, we have investigated the effect of altering SLIRP and SRA expression on programmed cell death. Following siRNA depletion of SRA, we observed a decrease in caspase activity in breast and cervical cancer cell lines while loss of SLIRP had the opposite effect. Similarly, targeted reduction of SRA in Hela cells rendered them less susceptible to staurosporine-induced apoptosis as measured by changes to nuclear morphology while reduced SLIRP promoted an earlier and more robust response to this agent. In complementary studies, both transient and stable over expression of SLIRP led to reduced apoptotic activity in a range of cell lines as measured by PARP, caspase 3 and 9 cleavage. These data suggest SRA and SLIRP are significant and opposing modulators of apoptosis and that assessment of their expression could provide important prognostic information for tumour therapy.

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SPECIFICITY OF ANDROGEN RECEPTOR ACTIVITY ON CHROMATIN IS DETERMINED BY MULTIPLE RECEPTOR DOMAINS

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Androgen receptor (AR) signalling exhibits distinct effects in multiple tissues and is critically important in both normal and disease states. Currently however, we understand little of how the AR engages with the cellular milieu and different ligands to mediate these effects. We have identified a set of clinically relevant gain-of-function AR mutations that collocate to a discrete region of the AR amino-terminal activation function five (AF5) located just upstream of the DNA binding domain in prostate cancer patients treated with complete androgen blockade. Comprehensive deletion mutagenesis of AF5 as well as the better characterised AF-1 and AF2 domains revealed that AF1 is mandatory for AR transactivation and protein stability, whereas AF5 and AF2 facilitate the magnitude and sensitivity of the receptor's transcriptional response to ligand. The structural integrity of AF5 appears to be essential for AR inter-domain communication via the N/C interaction and recruitment of p160 coactivators, but chromatin immunoprecipitation revealed that AF5 is not required for AR activity on a chromatin integrated reporter element. These analyses have defined two distinct classes of N/C deficient AR variant with divergent activity on chromatin. Moreover. the data indicate a complex interplay between p160 coregulator recruitment and N/C interaction via multiple receptor domains, and indicate that a model of AR activity based on interplay between receptor domains rather than the popular concept of modular domain action.. These studies provide important insights into the cellular and promoter specific actions of the AR and how this may relate to diseases such as prostate cancer...

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VINCLOZOLIN EXPOSURE *IN UTERO* INDUCES POST-PUBERTAL PROSTATITIS AND REDUCES DAILY SPERM PRODUCTION VIA A REVERSIBLE HORMONE REGULATED MECHANISM

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Environmental endocrine disrupting chemicals (EDCs) are defined as exogenous substances that alter function(s) of the endocrine system and consequently cause adverse health effects in an intact organism or its progeny. EDCs alter the action of gonadal steroid hormones by virtue of their anti-androgenic or estrogenic properties. The fungicide Vinclozolin is an anti-androgenic EDC that binds with high affinity to the androgen receptor (AR)1,2. The foetus is particularly sensitive to anti-androgenic insult because AR expression is essential for male reproductive tract development. Although exposure may be transient, effects are permanent and may lead to late life pathologies. Renewed concern about the effects of Vinclozolin re-emerged in 2005, when it was shown to have trans-generational effects on male rat fertility3.

The ability to reprogram the germ line resulting in trans-generational abnormalities has significant implications for the aetiology of reproductive diseases.

We previously reported in utero Vinclozolin exposure decreased anogenital distance (AGD) and induced prostatitis (prostate inflammation) in post-pubertal rats that was associated with down-regulation of AR and increased NFκB activation4. Based on these results and because Vinclozolin is an anti-androgen, we postulated, contrary to the trans-generational modifications described by others, in utero Vinclozolin exposure leading to post-pubertal (early onset) prostatitis was a reversible by androgen supplementation. To test our hypothesis we administered high-dose testosterone at puberty to determine if this would normalise AGD, abrogate prostatitis and restore testicular DSP; concurrently we examined DNA methyltransferase (Dnmt) expression.

Our results showed in utero Vinclozolin exposure leading to reduced AGD, development of prostatitis and reduced daily sperm production (DSP) was normalised by testosterone administration. Altered Dnmt expression, evident in Vinclozolin exposed tissues, was not normalised following testosterone treatment. These data prove Vinclozolin-induced male reproductive tract abnormalities are hormonally regulated and reversible, despite altered Dnmt expression.

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IDENTIFICATION OF MECHANISMS UNDERLYING PROSTATE TUMORIGENESIS DRIVEN BY ABERRANT ANDROGEN RECEPTOR SIGNALLING

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The androgen signalling axis is critical for all stages of prostate cancer, although the mechanism by which androgen signalling contributes to prostate tumorigenesis remains unclear. Enforced overexpression of an androgen receptor (AR) variant (AR-E231G) in mouse prostate results in development of metastatic prostate cancer in 100% of mice by 50 weeks old, whereas control mice overexpressing a different AR variant do not develop cancer1. At 12 weeks of age, AR-E231G mice develop prostatic intraepithelial neoplasia (PIN)-like lesions, but not cancer. In this study, we performed microarray analyses on prostate tissue from 12 week old AR-E231G mice to define global gene expression in the prostate before tumour development, and identified ~100 genes with altered expression compared to mice overexpressing a non-tumorigenic AR variant.

Almost half of the differentially regulated genes identified have known roles in carcinogenesis or androgen signalling pathways, including Rb1, Apc and several members of the kallikrein gene family. Downregulated genes included the tumour suppressors, Vangl2 and Apc. Key upregulated genes included the well-defined androgen regulated genes, Adm and Cited1, whose upregulation was verified by RT-qPCR. Using immunohistochemistry we demonstrated that both ADM and CITED1 proteins are expressed in human prostate cancer. At the cellular level, we confirmed androgen regulation of ADM RNA and protein in LNCaP human prostate cancer cells. Knockdown of ADM in LNCaP prostate cancer cells by siRNA significantly reduced cell proliferation and increased cell death, which is consistent with ADM overexpression being an initiating factor in prostate cancer.

ADM is an example of a gene identified in this study that has a potential role in the initiation of human prostate cancer. This highlights the potential of the AR-E231G model of aberrant androgen signalling as a novel tool to identify genes affecting all stages of prostate cancer, from initiation, to progression and development of metastatic disease.

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ESTRADIOL INDUCTION OF SPERMATOGENESIS IS MEDIATED VIA AN ERA AND NOT ERB MECHANISM INVOLVING NEUROENDOCRINE ACTIVATION OF FSH SECRETION

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Spermatogenesis is induced in gonadotropin-deficient *hpg* mice by both testosterone or non-aromatizable dihydrotestosterone (DHT). Surprisingly, estradiol (E2) induces spermatogenesis and increases blood FSH in *hpg* males if androgen receptor (AR) and estrogen receptors (ERs) are present (1). The mechanism and site (hypothalamus-pituitary *vs* testis) of this E2 effect remains unclear. We examined the mechanism of E2-induced spermatogenesis in *hpg* mice on an ERα (*hpg*/AERKO) or ERβ (*hpg*/BERKO) knockout or wild-type ER (*hpg*/WT) background. Littermates (n=5-14/group) 3-4 weeks old were treated (6 weeks) with E2 or DHT. In *hpg*/WT and *hpg*/BERKO, but not *hpg*/AERKO, E2 significantly increased testis and epididymal weight, but less than DHT, whereas DHT (but not E2) also increased seminal vesicles, liver and kidney weight. E2 increased FSH levels in *hpg*/WT and *hpg*/BERKO but not in *hpg*/AERKO mice. LH was not increased in any group. In stereological analysis, DHT and E2 increased spermatogonia and spermatocytes. DHT increased postmeiotic (spermatid) development whereas E2 postmeiotic effects were more variable and required ERα . In parallel studies, weanling (3 wk)

hpg/WT mice were administered subdermal pellets (IRA) containing selective ERα (16α-LE_2 15 & 45 mg/kg) or ERβ (8β-VE_2 150 & 450 mg/kg) agonists (Schering) for 6 weeks. ERα (but not ERβ) agonist increased testis and epididymal weight and elevated FSH (but not LH) concentration. We conclude that E2 induction of spermatogenesis is mediated via a central ERα neuroendocrine mechanism increasing circulating FSH which then allows combined FSH-AR spermatogenic actions (1) in the testis. As E2 is sufficient, but not necessary, to initiate mouse spermatogenesis and acts primarily via an extra-testicular site of action, androgen and estrogen effects on spermatogenesis differ in both mechanism and locus.

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(1) Lim et al Reprod Fertil Dev 20:861-870, 2008

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ELEVATED LEVEL OF INHIBIN-A SUBUNIT IS PRO-TUMOURIGENIC AND PRO-METASTATIC AND ASSOCIATED WITH EXTRACAPSULAR SPREAD IN ADVANCED PROSTATE CANCER

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The biological role of inhibin-α subunit (INHα) in prostate cancer (PCa) is currently unclear. A recent study associated elevated levels of INHα in PCa patients with a higher risk of recurrence. This prompted us to use clinical specimens and functional studies to investigate the pro-tumourigenic and pro-metastatic role of INHα. We conducted a cross-sectional study to determine a link between INHα expression and a number of clinicopathological parameters including Gleason score, surgical margin, extracapsular spread, lymph node status and VEGF-R3 expression which are well established prognostic factors of PCa. In addition, using two human PCa cell lines (LNCaP and PC3) representing androgen-dependent and androgen-independent PCa respectively, this study investigated the biological role of elevated levels of INHα in advanced cancer. Elevated expression of INHα in primary PCa tissues showed a higher risk of PCa patients being positive for clinicopathological parameters outlined above. Over-expressing INHα in LNCaP and PC3 cells demonstrated two different and cell-type specific responses. INHα-positive LNCaP demonstrated reduced tumour growth while INHα-positive PC3 cells demonstrated increased tumour growth and metastasis via the process of lymphangiogenesis. Gene array studies suggest that the pro-metastatic effect of INHα may be due to an alteration in the ERK/MAK pathway. This study is the first to demonstrate a pro-tumourigenic and pro-metastatic role for INHα associated with androgen-independent stage of metastatic prostate disease. Our results also suggest that INHα expression in the primary prostate tumour can be used as a predictive factor for prognosis of PCa.

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AUTOCRINE INHIBINS REGULATE IMMATURE MOUSE LEYDIG CELL BEHAVIOUR

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Inhibins are members of the Transforming Growth Factor-β (TGF-β) superfamily of pleiotrophic growth and differentiation factors. Inhibins were characterized originally as gonadally-derived molecules that acted in an endocrine manner to regulate pituitary hormone release. More recently, inhibin has been shown to antagonise activin and BMP action *in vitro* through binding to betaglycan, thereby providing a hypothesis for a local autocrine/paracrine role of inhibins in the tissues that produce them. The objective of the current study is to test this hypothesis by determining the potential autocrine actions of inhibins in TM3 Leydig cells. To determine whether key markers of differentiated Leydig cell function are regulated by autocrine inhibin action, quantitative real time PCR analysis was conducted using total RNA derived from TM3 Leydig cells which had been treated with 1) exogenous TGF-β superfamily members TGF-β1, TGF-β2, Activin A, or Inhibin A; or 2) a pan-specific TGF-β neutralizing antibody to eliminate endogenous TGF-β signaling; or 3) *Inha* shRNA to knockdown the expression of autocrine inihibins. This analysis revealed that TGF-β1, TGF-β2, or Activin A reduced the expression of *Inha* , *Cyp17a1*, and *Sf1* by ~50% ($p<0.05$). Neutralisation of autocrine TGF-β had no significant effect on steroidogenic gene expression although the neutralizing antibody did reverse the effects of exogenous TGF-β1 and TGF-β2. In contrast, knockdown of *Inha* reduced the expression of *Cyp11a1*, *Cyp17a1*, *StAR*, and *Sf1* by ~40-80% ($p<0.05$), presumably due to enhanced autocrine activin activity. These results were confirmed by luciferase reporter assays using a *Cyp17a1* reporter construct. In contrast, none of the various treatments significantly affected TM3 cell proliferation as determined by MTT and FACS assays. We conclude that inhibins, but not TGF-βs, act in an autocrine manner to regulate differentiated function in TM3 Leydig cells. Supported by the NH&MRC of Australia (RegKeys 338516; 241000; 441101; 388904).

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ASSOCIATION BETWEEN COMMON VARIANTS IN THE FTO GENE, TYPE 2 DIABETES AND MORTALITY

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Common variants in the FaT mass and Obesity-associated (FTO) gene are associated with body mass index (BMI). However, the contribution of the FTO gene to type 2 diabetes (T2D) risk is not clear. This study sought to examine the association between genetic variants within the FTO gene and the risk of T2D and mortality in a group of elderly men and women.

Six single nucleotide polymorphisms (SNP: rs1421085, rs1558902, rs1121980, rs17817449, rs9939609 and rs9930506) within the FTO gene were genotyped by Taqman assays in 696 men and 1180 women aged 60+ years who were participants in the Dubbo Osteoporosis Epidemiology Study. The men and women have been followed up for 18 years since 1989. T2D was ascertained by direct interview and confirmed by reviewing medical prescriptions. Mortality was ascertained by direct follow-up. Baseline assessment included anthropometric indices, lean mass, and fat mass (measured by DXA).

During the follow-up period, 67 men and 84 women were diagnosed with T2D. Men and women with T2D had significantly greater BMI, lean mass and fat mass than non-diabetic individuals. All 6 SNPs were associated with T2D risk in men, but not in women. The odds of T2D in men with the rs9939609 A allele was increased by 1.9-fold (95%CI: 1.3 to 2.7) after adjusting for obesity (BMI>30; odds ratio [OR] 3.1; 95%CI: 1.9 to 5.5). The proportion of T2D in men attributable to the rs9939609 SNP and obesity was 14% and 30%, respectively. Moreover, men carrying the rs9939609 A allele had a greater risk of mortality than men without the allele (OR 1.7; 95%CI: 1.1 to 2.7), independent of age, BMI and diabetic status.

Thus, these results suggest that common variation within the FTO gene is associated with increased risk of T2D and mortality in men, but not in women.

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ROLE OF MICRORNAS IN PLACENTAL PROGRAMMING OF INSULIN RESISTANCE

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Introduction: Placental restriction (PR) is a major cause of intrauterine growth restriction (IUGR), which is associated with adult-onset type 2 diabetes. This is due to insulin resistance and down-regulated expression of insulin signalling molecules in skeletal muscle in human, rats and sheep. MicroRNAs (miRs) are small non protein coding RNAs that can down-regulate of multiple target mRNAs. We hypothesised that PR and IUGR in the sheep alters expression of miRs in skeletal muscle of progeny, which target and alter insulin signalling expression and action, in lambs (44 days old) and adults (18 months old).

Methods: Placental growth in sheep was restricted by removal of the majority of endomentrial caruncles from non-pregnant Merino ewes, and hence placental size and function. Vastus lateralis was collected at post-mortem, miR expression analysed by Exiqon microarray v8.1. Bioinformatic analyses used to identify the predicted gene targets for each differentially expressed miR. Ingenuity Pathway Analysis (IPA) was used to identify metabolic and signalling and other pathways that might be affected.

Results: PR reduced skeletal muscle expression of miR-211 and miR-451 ($p<0.05$) in lambs overall and only miR-365 ($p<0.05$) in females and did not alter any in males. In adult sheep, PR increased miR expression in females only: miR-278, miR-376b, miR-175p, miR-142-3p, miR-21, miR-101 and miR-324-3p ($p<0.05$). Finally, PR reduced skeletal muscle miR expression in male progeny, regardless of age: miR-663, miR-711, miR-720, miR-612, miR-197, miR-409-5p and miR-769-3p ($p<0.05$). Some differentially expressed miRs were predicted to target mRNAs of proximal insulin signalling molecules, and other pathways; ERK/MAPK, GABA receptor and JAK/Stat signalling.

Conclusion: The present study shows that early life perturbation by PR can alter the expression of miRs in skeletal muscle of progeny and potentially lead to the altered expression of insulin signalling molecules and potentially other signalling pathways.

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RESISTANCE TO THE EFFECTS OF LEPTIN REPLACEMENT THERAPY OF IMMUNOLOGICAL ORIGIN IN CHILDREN WITH BERARDINELLI SEIP CONGENITAL LIPODYSTROPHY (BSCL)

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Objective: Leptin replacement has been introduced in the early 2000's for the treatment of the metabolic disorder induced by lipodystrophy and the very first results in adult diabetic patients lead to improvement of insulin sensitivity and reduction or cessation of the anti-diabetic treatment. A positive response was also recently reported in 6/7 non diabetic children with BSCL during a 4-month proof-of-concept trial which prompted us to hypothesize that in such patients leptin replacement therapy could improve or reverse the early complications of the disease.

Research design and methods: A second trial was implemented in 8 patients (6 boys, 2 girls, 5 to 16 years) for 28 months to test for the long-term efficiency and tolerance of such a therapy. Efficacy criteria were: decrease in serum TG, decrease in liver volume and increase in insulin sensitivity (≥30% each). The number of improved criteria defined the response as total (3/3), partial (1 or 2/3), or negative (0/3). Leptin doses were initially at 0.06 mg/kg/d and further increased up to 0.12 mg/kg/d in the case of lack of response. Anti-leptin antibodies were measured with a sensitive homemade radiobinding-assay. Neutralizing effect was assessed in vitro by measuring percent of pycnotic nuclei in primary culture of ofetal neurons incubated with an apoptotic agent (NMDA) and the patient serum and with or without leptin.

Results: By contrast to the first trial, a negative or partial response to treatment has been observed in the majority (5/8) of the patients even when leptin dosage was increased. A displaceable leptin binding was detectable in all patients after 2 months of treatment. At month 28, binding was higher in the patients with a negative response than in the total responders (62.4+/-28 vs 34.5+/-4.41 % respectively) and paralleled both the increase in leptin dosage and serum leptin concentrations contrasting with the lack of clinical response. Co-incubation of fetal neurons with serum from patients with negative response inhibits the neuroprotective effect of leptin.

Conclusion: Children with BSCL, who hardly secrete any leptin, develop an immunological long-term resistance to leptin therapy in relation with the production of neutralizing antibodies against leptin. This immunological reaction counteracts the previously reported beneficial effects of leptin administration in BSCL.

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HIGH CORTISOL RESPONSIVENESS MAY BE A MARKER FOR THE INNATE PREDISPOSITION TO BECOME OBESE

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Previous studies have demonstrated that sheep with relatively higher cortisol response to Synacthen (adrenocorticotropic hormone) have lower feed efficiency, indicating that these animals eat beyond the predicted requirement based on size and growth rate. We sought to determine whether differences in food intake in animals that have relatively high or low cortisol response to Synacthen display differences in the propensity to become obese. High and low responding female sheep were identified by administering a single iv injection of Synacthen (0.2 µg/ kg body weight) and measuring the area under the curve (AUC) of cortisol response over 1.5h (n= 40). The AUC of high responders (HR: n=5) was 198+/-6.3 and of low responders (LR: n=4) was 144.5+/-12.4 ($P<0.01$). To determine whether cortisol responsiveness predicted the propensity to develop obesity we characterised changes in body composition in two models, diet-induced obesity and seasonally-driven obesity. Body composition was characterised by dual energy X-ray absorptiometry. In addition, dietary preference was assessed by measuring intake of chaff or a high energy supplement. In June (a period of low appetite drive), chaff intake was similar in HR and LR animals, but intake of the high energy supplement was greater ($P<0.05$) in the LR group. Levels of adiposity were similar in LR and HR prior to and after dietary manipulation. In contrast, the HR displayed greater ($P<0.01$) adiposity during January, a period of high appetite drive. Regression analyses demonstrated that the % adiposity was correlated to cortisol responsiveness in obese animals (after dietary supplementation and in January), but not in animals of normal body weight. The relatively higher adiposity in HR under conditions of high appetite drive suggests that cortisol responsiveness may be a marker for the propensity to develop obesity. This hypothesis requires testing in a broader context in relation to age, sex and other factors.

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MONOTHERAPY WITH LIRAGLUTIDE, A ONCE-DAILY HUMAN GLP-1 ANALOGUE, PROVIDES SUSTAINED REDUCTIONS IN HBA_{1C}, FPG, AND WEIGHT COMPARED WITH GLIMEPIRIDE IN TYPE 2 DIABETES: LEAD-3 MONO 2-YEAR RESULTS

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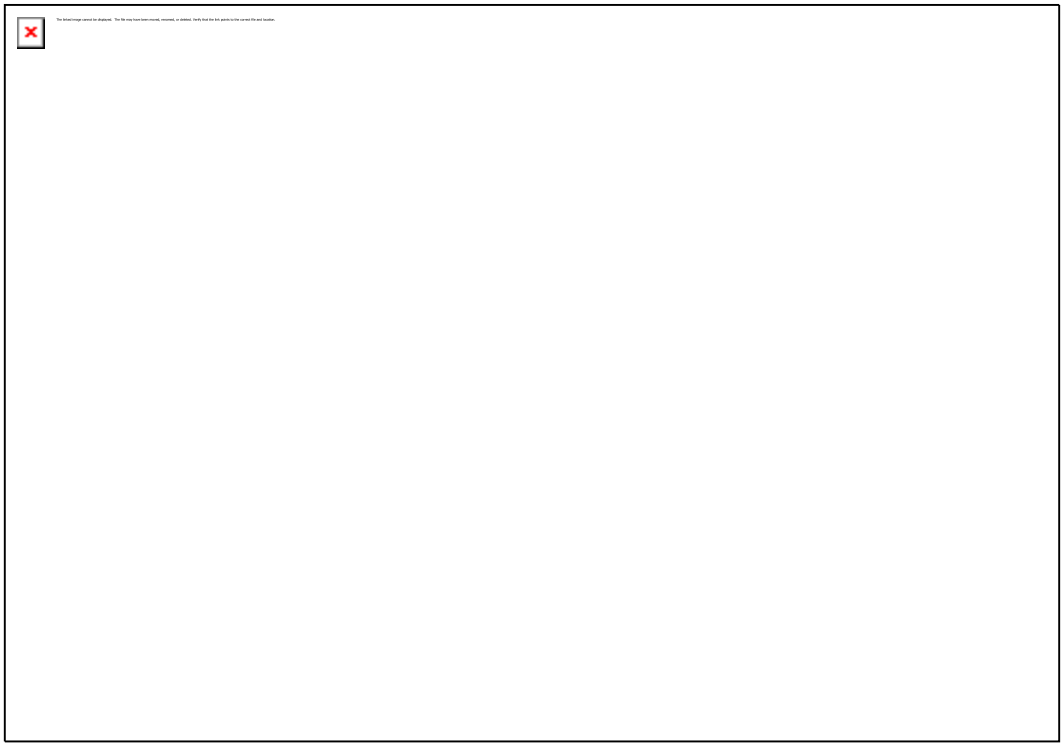
Efficacy and safety of liraglutide (1.8 and 1.2mg, QD) were compared to glimepiride (8mg QD) during the LEAD-3 trial, a 1-yr randomised, double-blind trial followed by an open-label extension. Ninety percent (440 subjects) of 1-yr completers entered the extension, and 321 (73%) of them completed 2 yrs (2-yr completers: mean age 54 yrs, BMI 33 kg/m^2, HbA_{1C} 8.2%; median diabetes duration 3.3 yrs, prior diet/exercise only [36%] or 1 OAD [64%]). Two yrs of liraglutide monotherapy (1.8 and 1.2mg) reduced HbA_{1C} more than glimepiride (ANCOVA, p=0.0016 and p=0.0376) and more subjects reached HbA_{1C} <7.0% at 2 yrs (p=0.0054 and p=0.0269). Weight loss with liraglutide and weight gain with glimepiride were sustained (p<0.0001). Hypoglycemia (<3.1mmol/L) was > 6 times less frequent with liraglutide 1.8 and 1.2mg vs glimepiride (p=0.0001 and p<0.0001). Between-treatment HbA_{1C} differences were comparable between ITT (LOCF) and 2-yr completer analyses; ITT (LOCF) analyses also showed significant reductions in HbA_{1C}, FPG, weight, and hypoglycaemia with liraglutide vs glimepiride. In conclusion, 2 yrs of liraglutide monotherapy provides significant and sustained improvements in glycaemic control and bodyweight compared with glimepiride monotherapy, with lower risk of hypoglycemia.



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TETRAHYDROCANNABINOL (THC) ATTENUATES WEIGHT LOSS IN AN ACTIVITY BASED MODEL OF ANOREXIA NERVOSA

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Activity-based anorexia (ABA), an animal model of anorexia nervosa (AN), involving scheduled feeding and voluntary exercise (running wheel activity (RWA)) leads to hypophagia, dramatic body weight loss, and ammenhoria. It has long been recognised that both externally introduced cannabinoids and activation of an endogenous "endocannabinoid" system can elevate food intake, particularly of high energy, palatable foods. However, the potential for these substances to ameliorate weight loss in AN or in the animal ABA model is poorly understood. In the present experiments, the effect of chronic (THC) treatment on ABA rats was investigated. The ABA model involves ad lib access to food (either standard chow or high fat) for 90 minutes daily with continuous access to running wheels. Female Sprague-

Dawley rats (N=30) were treated with vehicle or THC (2mg/kg i.p.) daily for 5-7 days and in two separate studies allowed access to 1) standard laboratory chow or 2) both chow and palatable high fat food. THC treatment in both studies showed a significant attenuation of ABA while producing only a transient stimulation of feeding. The high fat diet in itself attenuated ABA in a manner indistinguishable from THC treated chow fed animals. However, THC and a high fat diet in combination produced what appeared to be a synergistic attenuation of weight loss that was significantly greater than weight loss in either vehicle treated high fat or THC treated chow animals. Measurement of abdominal fat in each of these groups supported the efficacy of the combined high fat diet/THC treatment in ameliorating weight loss in ABA. These data highlight the potential of cannabinoids to attenuate weight loss seen in ABA, particularly when combined with exposure to highly palatable foods.

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THE ROLE OF AN ENDOGENOUS INHIBITOR OF CALCINEURIN IN THE REGULATION OF GLUCOSE HOMEOSTASIS

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Calcineurin (CaN) is a protein phosphatase important in the regulation of transcription and in protein phosphorylation associated with cell signaling. Treatment of post-operative transplant patients with immunosuppressants such as cyclosporine and FK-506, which are CaN inhibitors, regularly induce diabetes (Weir and Fink, 1999) . This occurs via inhibition of the CaN/NFAT transcription pathway which regulates pancreatic β-cell growth, proliferation and insulin secretion (Heit et al., 2006) . We are investigating the effect of increased expression of a gene known to endogenously inhibit CaN, and its possible role in the pathogenesis of diabetes. Transgenic mice with a universal over-expression of this gene were generated and used for this study. mRNA expression levels were examined using quantitative real-time RT-PCR. Expression of our gene of interest increased 2.5 fold ($p<0.05$) when islets were exposed *in vitro* to 16.7 mM glucose for 6 days. In islets of transgenic mice, genes such as those mutated in hereditary forms of monogenic type 2 diabetes (MODY) and others that regulate β-cell survival, proliferation and insulin production were downregulated. Immunohistochemical analysis of pancreatic islets revealed that transgenic mice have a 70% reduction in islet area (n=4) at 100 days. *In vivo* studies indicate that transgenic mice develop age-dependent diabetes characterized by increased fasting blood glucose levels of 5.8±0.3 mmol/L (n=9) at 60 days old compared to 4.2±0.2 mmol/L (n=9) in age and sex-matched wild-type mice ($p<0.05$). This difference in fasting blood glucose levels progressively increases with age. These changes are not due to differences in body weight or increased insulin resistance in the transgenic mice. Glucose tolerance, measured by injecting 2mg glucose/g body weight, is also reduced in transgenic mice, with glucose values reaching peak levels of 27.5±1.4 mmol/L (n=5) after 60 minutes compared to 19±1.3 mmol/L (n=5) in wild-type mice ($p<0.01$). Our findings highlight a novel role of our gene of interest in regulating glucose homeostasis, expression of major β-cell regulatory genes and in islet growth. As this gene is also up-regulated by chronic high glucose exposure, our findings provide the exciting proposition that his gene may be involved in the β-cell failure and hypoinsulinemia that occurs in the later stages of type 2 diabetes.

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MATERNAL FOLIC ACID SUPPLEMENTATION IN THE RAT IMPROVES INSULIN DISPOSITION IN ADULT MALE OFFSPRING BUT IMPAIRS THAT OF FEMALE OFFSPRING

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Background: Exposure of pregnant women to high folic acid intake is common and likely to increase. The long-term effects of maternal folic acid supplementation (FAS) throughout pregnancy for progeny in humans or other species have been little studied. We have recently shown that maternal FAS in the rat improve glucose tolerance in adult offspring, but improve insulin sensitivity in males only while impairing it in females. We hypothesised that maternal FAS may also affect insulin secretion and disposition (secretion adjusted for sensitivity) in adult offspring and contribute to their improved glucose control, especially in females.

Methods: Pregnant rats were fed a control (2mg folic acid/kg diet) or FAS (6mg/kg diet) diet from two weeks before and throughout pregnancy. Glucose tolerance was assessed by intraperitoneal glucose tolerance test (IPGTT), insulin secretion from plasma insulin during IPGTT and insulin sensitivity by an IP insulin tolerance test in offspring at 3 months of age (n=8-10 males, n= 8-10 females in each treatment group). Insulin secretion adjusted for sensitivity was calculated as the product of these, termed insulin disposition.

Results: Maternal FAS reduced fasting blood glucose ($p=0.056$) and improved glucose tolerance ($p<0.044$) in adult offspring. FAS also altered plasma insulin ($p=0.008$) and plasma insulin:glucose ($p=0.005$) during IPGTT, reducing these in offspring. FAS tended to increase maximal insulin disposition in male offspring ($p=0.094$), but reduced both basal ($p=0.046$) and maximal ($p=0.05$) insulin disposition in female offspring.

Conclusion: Maternal FAS in normal pregnancy improves glucose control in adult male offspring in part by improving their insulin secretory capacity. In contrast, FAS improves glucose control in adult female offspring, despite impairing their insulin secretory capacity. Whether these outcomes persist or amplify with increasing age, with adverse consequences for glucose homeostasis in females longer term remains to be determined.

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MAP KINASE PATHWAYS ARE INVOLVED IN IGF-INDEPENDENT, IGFBP-6-INDUCED RHABDOMYOSARCOMA CELL MIGRATION

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A family of six high-affinity IGF binding proteins (IGFBPs) plays an important role in modulating IGF activities. Among them, IGFBP-6 is a relatively specific IGF-II inhibitor. Recently, using a non-IGF binding mutant of IGFBP-6 (mIGFBP-6), we reported that IGFBP-6 promotes RD rhabdomyosarcoma cell migration in an IGF-independent manner (1). We further investigated signaling pathways, particularly MAP kinase (Erk, p38 and Jnk) pathways, that may be involved in this process. Inhibiting Erk1/2 or p38 MAPK activation by specific inhibitors (10 μM PD98059 or SB203580 respectively) prevented mIGFBP-6-induced cell migration in both RD and Rh30 rhabdomyosarcoma cell lines, whereas a Jnk inhibitor had no effect. mIGFBP-6 induced transient p38 phosphorylation in RD cells but not in Rh30 cells. Erk1/2 is constitutively activated in both cell lines, and mIGFBP-6 induced further transient Erk1/2 phosphorylation in Rh30 but not RD cells. In contrast, mIGFBP-6 had no effect on Akt or NF-κB p65 phosphorylation. Furthermore, selective inhibition of p38 appeared to decrease mIGFBP-6-induced Erk1/2 activation in Rh30 cells, whereas inhibiting Jnk (10 μM SP600125), Akt (10 μM LY294002) or NF-κB (10 μM BAY 11-7082) had no effect. Surprisingly, mIGFBP-6 induced Jnk1 phosphorylation in Rh30 cells, an effect prevented by Erk1/2 and p38 inhibitors. These data suggest that Erk1/2 and p38 MAPK pathways are involved in IGF-independent IGFBP-6-induced rhabdomyosarcoma cell migration, and that cross-talk between MAPK pathways may play an important role in this process.

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HEDGEHOG SIGNALLING IMPLICATED IN STROMAL-MEDIATED LINEAGE COMMITMENT OF ADULT EPITHELIAL CELLS

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The ability of stroma to reprogram adult stem cells (SCs) has significant implications for regenerative medicine as it highlights the importance of placing these cells in the correct stromal niche to avoid causing an undesired cellular phenotype, and possibly even a disease pathology. It is known that signals from the embryonic prostatic stroma (derived from urogenital sinus mesenchyme, UGM, endodermal tissue) can direct the differentiation and lineage commitment of embryonic SCs[1,2], and redirect the lineage commitment of adult SCs housed within other endodermally derived epithelial tissues[3]. To determine the spectrum of tissues that developmental stroma is capable of reprogramming, we investigated whether UGM could redirect the lineage commitment of adult SCs from a different developmental germ lineage and what signalling pathways were involved. Using the technique of tissue recombination, UGM (endoderm) was recombined with adult mammary gland epithelium (ectoderm) and grafted under the renal capsule of a host immune deficient mouse for one month. The resulting epithelium was analysed for prostate and mammary specific markers, and screened for expression levels of key genes from signalling pathways important for prostate, mammary development. Immunohistochemically, the recombined epithelium exhibited a chimeric phenotype of both prostate and mammary glands: androgen receptor staining, not normally in breast tissue but abundant in the prostate, was observed; as were both prostate and mammary specific α-actin smooth muscle staining and localisation patterns. Gene expression arrays revealed that Hedgehog (Hh) signalling pathway downstream target genes CyclinD1, Foxa2, E-cadherin, and Patched1 were upregulated in the recombined epithelium compared to mammary and prostate tissue controls. These data show that stroma can reprogram the lineage commitment of adult epithelial SCs across germ lineages, via a process involving Hh signalling. Further studies will be required to functionally test the involvement of the Hh pathway, and to determine its role in lineage commitment.

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CHIP-SEQUENCING OF STEROID RECEPTORS IN BREAST CANCER

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The growth of many breast cancers is initially dependent on hormonal signals, and primarily on the action of estrogens and progestins through two related steroid nuclear receptors (SRs), estrogen receptor alpha (ER a) and progesterone receptor (PGR) respectively. Emerging evidence suggests that the biological program mediated by ER a in the breast is shaped by androgens (i.e. testosterone) through the related androgen receptor (AR), and that this crosstalk may underpin responses to anti-estrogen therapy. To address in detail the mechanisms of crosstalk between these three steroid receptors, we assessed the endogenous transcriptional capacity of AR, ER a and PGR on ~160 hormone responsive promoter constructs in a set of human breast cancer cell lines. That analysis revealed transcriptional crosstalk rates of 25%, deriving from both the cell-specific profile of coregulators and an unexpected plasticity in DNA response element

recognition. Illumina expression microarray analysis in breast cancer ZR-75-1 cells found that each receptor exerts a uni-directional effect on 25-30% of another receptors responsive loci. We undertook unbiased chromatin capture analysis with AR, ER a and PGR in ZR-75-1 cells using next-generation chromatin immunoprecipitation-sequencing (ChIP-seq). This approach captured >50 million 35bp sequence tags representing DNA regions enriched for occupancy by the three SRs. Development of bioinformatic approaches has allowed us to define the genome wide complement of high-confidence SR binding sites to within short 100-500bp regions. For example, we identified 65959 low, 11169 medium and 3954 high stringency ER a binding sites in the ZR-75-1 genome. At low stringency, the crosstalk between AR and ER a was 11.7%, but increased to 19.4% for high stringency sites. These findings demonstrate that steroid receptors share a high proportion of genomic target loci, and provide evidence for direct crosstalk signalling pathways. These studies are providing unprecedented detail on the role, mechanisms and outcomes of SR action in breast cancer .

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EFFECTS OF RESTRICTION OF PRENATAL AND POSTNATAL GROWTH ON SKELETAL MUSCLE DEVELOPMENT

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Fetal growth restriction results in impaired organ development and programs disease in adulthood. We investigated the developmental timeline of skeletal muscle following growth restriction in rats. We also used cross-fostering to determine the impact of prenatal and postnatal nutritional restraint on skeletal muscle.

Bilateral uterine vessel ligation (*Restricted*) or sham (*Control*) surgery was performed on day 18 of gestation in WKY rats. Offspring were killed at gestational day 20 or 1, 7 or 35 days after birth. For the cross-foster study, *Control* and *Restricted* pups were cross-fostered onto either a *Control* or *Restricted* mother one day after birth and killed at 7 days. Hindlimb muscles were pooled within litters according to sex. However, at day 35 gastrocnemius muscle was collected from individual offspring. PGC1α, MRF4, MyoD and myogenin mRNA expression was examined by real-time PCR (n=7-10).

Restricted offspring were smaller than *Controls* at all ages ($P<0.05$). However, *Restricted* pups cross-fostered onto a *Control* mother were not different to *Control* at day 7. PGC1α, MRF4 and MyoD mRNA was lower at gestational day 20 and increased such that expression was highest at day 35 ($P<0.05$). Myogenin mRNA peaked at day 1 with expression lowest at day 35 ($P<0.05$). At day 35, PGC1α, MRF4, MyoD and myogenin mRNA was higher in females than males ($P<0.05$) and was the case for PGC1α and myogenin at day 7 ($P<0.05$). There was not effect of growth restriction on PGC1α, MRF4 and myogenin mRNA. At day 7 MyoD was higher in *Restricted* compared to *Control* and was intermediate in *Restricted* females but not males fostered onto a *Control* mother.

In conclusion, myogenic regulatory factors have distinct developmental profiles with clear sex differences emerging at 7 and 35 days after birth. These sex differences highlight the perinatal implications for programming of adult metabolic disease and muscle function.

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THE REGULATION OF AROMATASE IN THE BREAST BY THE LKB1/AMPK PATHWAY PROVIDES A LINK BETWEEN OBESITY AND BREAST CANCER IN POSTMENOPAUSAL WOMEN

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It is now well established that obesity is linked to an increased risk of cancers such as colon and breast. Given the obesity problem worldwide, tens of millions more women may contract breast cancer in their senior years than was previously thought. After menopause, it is the local production of oestrogens within the breast adipose and catalysed by the aromatase enzyme that is believed to be responsible for the increased proliferation of breast cancer cells.

We have demonstrated that the LKB1/AMPK pathway is inhibitory of aromatase expression in primary human breast adipose stromal cells by inhibiting the nuclear entry of CRTC2, a CREB co-activator. Factors produced in obesity, such as leptin, and by tumours, such as PGE2, were shown to inhibit LKB1 expression and activity and cause the nuclear translocation of CRTC2, resulting in the increased expression of aromatase. Conversely, a factor produced in lean individuals, adiponectin, and the drug AICAR, known to activate AMPK, inhibited the PGE2-mediated expression of aromatase.

Metformin, an oral anti-diabetic drug, is believed to act primarily by stimulating AMPK. In the present study, we aimed to examine the effect of metformin on aromatase expression and on the LKB1/AMPK pathway within the breast. Primary human breast adipose stromal cells obtained from breast reduction surgeries were treated with increasing amounts of metformin and aromatase transcript expression was quantified. Interestingly, 24-hour treatment resulted in the dose-dependent inhibition of aromatase expression. LKB1 activity was measured by examining the phosphorylation of AMPK and consistent with the proposed role of LKB1/AMPK in aromatase expression, metformin caused a significant increase in AMPK phosphorylation.

In summary, this study identifies the LKB1/AMPK/CRTC2 pathway as a regulator of aromatase expression within the breast and suggests that an anti-diabetic drug that targets this pathway could be used to treat obesity-related postmenopausal oestrogen-dependent breast cancer.

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COMBINING A HSP90 INHIBITOR WITH OTHER AGENTS THAT ABROGATE ANDROGEN SIGNALLING SYNERGISTICALLY INDUCES DEATH OF PROSTATE CANCER CELLS

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Heat shock protein 90 (Hsp90) inhibitors have exhibited considerable potential for inhibiting the growth of solid tumours as Hsp90 inhibition results in degradation of Hsp90 client proteins, including many survival factors and oncogenes. Hsp90 is an important target for prostate cancer as the androgen receptor (AR), the key mediator of prostate cancer cell growth and survival, is a Hsp90 client protein. The Hsp90 inhibitor 17-allylamino-demethoxygeldanamycin (17-AAG) has been shown to have profound effects on AR function and stability in prostate cancer cells, resulting in inhibition of cell growth *in vitro* and *in vivo*. In this study, we investigated whether combining 17-AAG with other agents that inhibit AR expression or function could result in enhanced AR blockade and suppression of prostate cancer cell growth. We demonstrate that combinations of low sub-effective doses of 17-AAG with the AR antagonist bicalutamide or the histone deacetylase inhibitor vorinostat synergistically inhibit growth and induce caspase-dependent death of androgen-dependent LNCaP prostate cancer cells. Interestingly, the combinations do not alter steady state protein levels of the AR or other Hsp90 client proteins HER2, AKT or RAF1. These combinations were not effective in androgen-independent PC-3 prostate cancer cells, and the low doses of these agents had no effect when used alone in either LNCaP or PC-3 cells. Collectively, these results demonstrate that combining AR-targeting agents, at low doses that are ineffective when given alone, synergistically enhance growth suppression and death of AR-dependent prostate cancer cells. As prostate cancer cells are typically AR-dependent at all stages of progression, and have a highly primed and sensitized Hsp90 chaperone network, this combinatorial approach offers the exciting potential to provide a more specific therapeutic for men with prostate cancer.

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BEING BORN SMALL REDUCES THE NUMBER OF CARDIOMYOCYTE NUCLEI WHICH CAN BE INCREASED BY IMPROVING POSTNATAL NUTRITION AND GROWTH

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Cardiomyocytes cease proliferating soon after birth when they become terminally differentiated. A reduced complement of cardiomyocytes can adversely affect the functional and remodelling capacity of the heart. We aimed to determine whether uteroplacental insufficiency and fetal growth restriction reduces cardiomyocyte nuclei number in hearts of male rats, and if so, whether this can be overcome by restoring early postnatal nutrition during lactation. We studied male offspring from mothers that underwent bilateral uterine vessel ligation (Restricted) to induce intrauterine growth restriction or sham surgery (Control) on day 18 of gestation. Control and Restricted pups were cross-fostered onto Control (normal lactation) or Restricted (impaired lactation) mothers 1 day after birth. At 7 days cardiomyocyte nuclei number was determined stereologically. Cardiac mRNA expression was quantified (real-time PCR). There was a reduction ($p=0.02$) in body weight and absolute, but not relative, heart weight of Restricted-on-Restricted offspring on postnatal day 7. Growth restriction was accompanied by a 28% reduction ($p<0.05$) in total cardiomyocyte nuclei number. Providing a normal lactational environment to restricted offspring (Restricted-on-Control) improved postnatal growth such that body and heart weights were not different compared to Control-on-Control. Improved nutrition and growth was associated with a restoration of cardiomyocyte nuclei number to that in Control-on-Control. There were no differences in cardiac mRNA expression for growth factors (*Igf1*, *Igf1r*, *Igf2*) or differentiation/maturation markers (*Gata4*, *Anp*, *Myl2*, *Myh7*) across groups. All cross-fostering groups had increased cardiac mRNA expression of At1aR and At1bR compared to Control-on-Control ($p<0.05$) associated with altered cardiac growth. Upregulation of cardiac *Bcl2* and *Cmyc* suggests a compensatory increase in proliferation and reduction in apoptosis in cross-foster groups. Growth restriction due to uteroplacental insufficiency adversely impacts on cardiomyocyte nuclei number. Improvement of lactational nutrition, at a time when the cardiomyocytes are still undergoing proliferation, prevents the deficit in cardiomyocyte number associated with growth restriction.

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BETAGLYCAN REGULATES TGF-BETA SUPERFAMILY ACTION IN HUMAN GRANULOSA TUMOUR CELLS

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Granulosa cell tumours (GCTs) account for approximately 5% of total ovarian cancer incidences. We have recently reported that reduced betaglycan expression may contribute to the malignant behaviour of GCTs, increasing their ability to migrate and invade extracellular matrix. In two human GCT cell lines, the COV434 and KGN, we demonstrated that inhibins and TGF-beta both acted via betaglycan to regulate different aspects of migration and invasion (Bilandzic et al., 2009, *Mol Endo* 23(4):539–548) . The objective of the current study was test the hypothesis that the presence of betaglycan affected the balance of TGF-beta superfamily signalling in these cells, thus altering their capacity to migrate and invade. Following treatment with exogenous TGF-beta2, *INHA* gene knockdown, or a combination of the two, total cell lysates were extracted from GCT cells which had been stably transfected with full-length wildtype betaglycan or a vector control. The relative levels of activation of specific pathways were determined by western blot analyses using activation-specific antibodies against

SMAD2, SMAD3, and SMAD1/5. This analysis revealed that the COV434 and KGN cells exhibit different profiles of activation for each of the SMAD molecules. The presence of betaglycan resulted in constitutive SMAD3 activation in KGN cells and significantly increased the activation of SMAD2 in response to TGF-beta2 in both KGN and COV434 cells. Furthermore, knockdown of *INHA* expression further enhanced TGF-beta2 mediated SMAD2 activation in the betaglycan-expressing cells. Collectively, our data indicate that the presence of betaglycan on GCT cells impacts on both inhibin- and TGF-beta-mediated GCT behaviours. Furthermore, the elimination of autocrine inhibin actions in the GCT cells enhanced TGF-beta2 stimulated SMAD2 activation in a betaglycan-dependent manner, suggesting that the presence of betaglycan on the surface of GCT cells counterbalances inhibin- and TGF-beta mediated signalling. Supported by: the NHMRC of Australia (RegKeys 338516; 241000; 441101; 388904).

(1) Bilandzic et al., 2009, Mol Endo 23(4):539–548

SRB ORALS FROM JOINT ESA-SRB SESSIONS

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IMMUNE-DEVIATING CYTOKINES DETERMINE THE MATERNAL T CELL RESPONSE DURING PREGNANCY AND TOLERANCE OR REJECTION OF THE CONCEPUTS

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In healthy pregnancies the maternal immune system establishes paternal antigen-specific tolerance allowing survival of the semi-allogeneic conceptus. The cytokine environment is a key factor in determining the phenotype of antigen-specific lymphocytes, influencing the development of either cytotoxic or tolerogenic cells. We hypothesized that the cytokine environment at the time of priming to paternal antigens influences the phenotype of the maternal T cell response and pregnancy outcome. Transgenic Act-mOVA male mice expressing chicken ovalbumin (OVA) ubiquitously provided OVA as a model paternal antigen. OVA is present within the semen of Act-mOVA mice and is inherited and expressed by the conceptus tissue. OVA-reactive CD8+ OT-I T cells were activated with OVA in the presence of various immune-deviating cytokines *in vitro*, before transfer at 3.5 dpc to C57Bl/6 (B6) females gestating OVA-expressing fetuses. Pregnant mice received either naïve OT-I T cells, cytotoxic OT-I T cells stimulated *in vitro* in the presence of IL-2 or OT-I T cells stimulated *in vitro* in the presence of TGFβ1 and IL-10, two factors present in the uterus and associated with immune tolerance. Immunhistochemistry was utilized to demonstrate that OT-I T cells infiltrate into the implantation site. Cytotoxic OT-I T cells caused fetal loss, while OT-I T cells activated *in vivo* or *in vitro* with TGFβ1 and IL-10 did not cause fetal loss. Additionally, cytotoxic OT-I T cells did not affect B6 x B6 matings, demonstrating the antigen-specific nature of the T cell-mediated fetal loss. Collectively these experiments show that maternal antigen-reactive T cells activated *in vivo* in the cytokine environment of the mated uterus are tolerogenic, not cytotoxic, and implicate TGFβ1 and IL-10 as key elements of that environment. We conclude that the cytokine environment at the time of priming to paternal antigens influences the T cell phenotype and impacts upon maternal immune tolerance and fetal survival.

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SPATIAL REGULATION OF APC^{CDH1} INDUCED CYCLIN B1 DEGRADATION MAINTAINS GV ARREST IN MOUSE OOCYTES

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Within the mammalian ovary, oocytes remain prophase I arrested until a hormonal cue triggers meiotic resumption. The E3 ubiquitin ligase, Anaphase-Promoting Complex with its co-activator Cdh1 (APC^{Cdh1}) is known to be essential for this process by promoting cyclin B1 degradation via the 26S proteasome. Cyclin B1 is the regulatory subunit of Maturation-Promoting Factor, forming a heterodimer with CDK1, which is essential for nuclear envelope breakdown (NEB). Here we describe the intracellular partitioning that would explain how Cdh1 activity is fine-tuned, such that cyclin B1 is maintained at sufficient levels to allow oocytes to resume meiosis but not so high as to cause premature meiosis re-entry. Using RT-PCR we detected only one splice variant in mouse oocytes, Cdh1α, which possessed a nuclear localisation signal. By immunofluorescence we confirmed the nuclear location of Cdh1 and degradation machinery components including essential subunits of the APC and 26S proteasome. In all systems studied, cyclin B1 shuttles between the cytoplasm and nucleus, with nuclear localisation occurring just before NEB. We reasoned therefore that the nuclear localisation of the APC^{Cdh1} and 26S proteasome would aid in maintaining low nuclear levels of cyclin B1. Using two GFP-coupled cyclin B1 mutants, which differed in their intracellular location we found that nuclear accumulation of cyclin B1 was necessary in order for it to promote meiotic resumption because over-expression of nuclear-cyclin B1 accelerated entry into meiosis, whereas cytoplasmic-cyclin B1 did not. However, in milrinone-arrested GV oocytes rates of nuclear-cyclin B1 degradation were 5 fold higher than cytoplasmic-cyclin B1. Therefore we conclude that in oocytes, an increase in the nuclear-cytoplasmic ratio of cyclin B1 is an essential step in meiotic resumption, and that nuclear APC^{Cdh1} activity guards against early meiotic resumption, until the degradation machinery is overwhelmed by cyclin B1 translocation. Supported by NHMRC (Grant 569202) to KTJ.

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EPIDERMAL GROWTH FACTOR RECEPTOR/MAPK3/1 PATHWAY CROSS-TALK ENABLES GROWTH DIFFERENTIATION FACTOR 9 TO SIGNAL THROUGH SMAD2/3 IN MOUSE GRANULOSA CELLS

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Oocyte-secreted growth differentiation factor 9 (GDF9) plays a critical role throughout folliculogenesis. It has been shown to control many functions of granulosa cells, including gene expression, steroidogenesis and proliferation. This study investigates the cellular requirements that allow GDF9 to act on granulosa cells. Our results showed that GDF9 (20 ng/ml)-stimulated mouse granulosa cells ^{3}H-thymidine incorporation was inhibited by a type 1 receptor Alk4/5/7 inhibitor (SB431542, 5 μM), by an epidermal growth factor (EGF) receptor inhibitor (AG1478, 5μM) and a MEK1 inhibitor (U0126, 10 μM). Interestingly, activin A- and TGFβ-stimulated ^{3}H-thymidine incorporation shared similar inhibitor sensitivity. Moreover, when denuded oocytes were used as the mitogenic agent, SB431542, AG1478 and U0126 all prevented the increase in ^{3}H-thymidine incorporation. Oocyte-stimulated ^{3}H-thymidine incorporation in secondary follicles

and cumulus-oocyte complexes were also sensitive to Alk4/5/7, EGF receptor and MEK1 inhibition. Basal and EGF-stimulated levels of phopho-MAPK3/1 were inhibited by using the EGF receptor inhibitor, but were not affected by inhibition of Alk4/5/7 or by adding GDF9 in granulosa cells. Using granulosa cells transfected with a SMAD3-luciferase reporter construct, GDF9-stimulated SMAD3 response could be inhibited by Alk4/5/7, EGFR and MEK1 inhibitors. Genes involved in cumulus cells expansion (Ptx3 and Has2) were upregulated in granulosa cells by co-culturing with denuded oocytes and that upregulation was inhibited by Alk4/5/7 as well as by EGF receptor inhibition. These results suggest that TGFβ superfamily members signalling through Smad2/3 share a common requirement of EGF receptor-dependant phospho-MAPK3/1 throughout folliculogenesis.

These results strongly suggest that, apart from its role in the transmission of the ovulatory LH signal within the ovarian follicle, EGF receptor pathway might serve as modulators of GDF9 action on granulosa cells. Hence the interaction between endocrine and oocyte signalling may be mediated at the level of MAPK and Smad2/3 cross-talk in granulosa cells.

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FATTY ACID OXIDATION IS ESSENTIAL FOR OOCYTE DEVELOPMENTAL COMPETENCE

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Oocyte lipid composition and developmental competence are influenced by dietary fat yet whether lipids are metabolised by the oocyte or essential for subsequent embryo development is largely unexplored. Fatty acid oxidation (FAO) is largely overlooked as an energy source for the oocyte, despite generating several-fold more energy than glucose oxidation. FAO requires the rate-limiting enzyme carnitine palmitoyltransferase-1 (Cpt1) and the metabolite Carnitine to shuttle fatty acids into mitochondria for energy production. Analysis of Cpt1 mRNA during oocyte maturation showed that Cpt1 expression was hormonally induced ($p<0.05$) in the cumulus oocyte complex (COC), peaking at 10h following ovulatory hCG treatment. In contrast, Cpt1 was not hormonally regulated in granulosa cells ($p>0.05$). To investigate the role of Cpt1-mediated FAO during oocyte maturation we measured FAO in oocytes in the presence and absence of Carnitine and inhibited FAO to determine its importance for oocyte developmental competence. Levels of FAO in COCs were assessed as metabolism of the fatty acid 3H-palmitate. During oocyte maturation there was a 2.1-fold increase ($p<0.0001$) in FAO compared to immature COCs. Carnitine supplementation led to a further 3.7-fold increase ($p<0.001$), while inhibition of Cpt1 with Etomoxir resulted in a 6.5-fold decrease ($p<0.0002$) in FAO during oocyte maturation. FAO inhibition had no effect on cumulus expansion. However inhibition of FAO during oocyte maturation followed by IVF and embryo culture in the absence of inhibitor, resulted in significantly decreased numbers of embryos developing 'on time' ($p<0.002$).

This is the first identification of hormonal induction of Cpt1 and Cpt1 mediated FAO in the COC during oocyte maturation. Further, the results demonstrate that oxidation of fatty acids by the oocyte is essential for oocyte developmental competence and can be modulated by Carnitine. These findings provide a potential mechanism by which dietary fat, obesity or metabolic disorders including CPT deficiency lead to infertility.

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MUTANT STEROIDOGENIC FACTOR-1 FROM PATIENTS WITH DISORDERS OF SEX DEVELOPMENT SHOW REDUCED ACTIVATION OF THE TESTIS-SPECIFIC ENHANCER OF *SOX9*

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The orphan nuclear hormone receptor Steroidogenic Factor 1 (SF1; *NR5A1*) is expressed throughout hypothalamic, pituitary, gonadal and adrenal tissues. Naturally occurring human mutations combined with mouse knockout models have revealed a critical role for SF1 as a transcription factor at multiple stages during gonadal development and during development of the adrenal.

Missense mutation or truncation to SF1 in XY humans cause Disorders of Sex Development (DSD) with variable phenotypes. The precise mechanisms of SF1 action that fail in human DSD are not fully determined. This work aimed to utilise naturally occurring DSD-causing mutations in SF1 to increase our understanding of the sex determining function of SF1 in the developing male gonad. Recent work by others (1) identified SOX9 as a key target gene of SF1 during testis determination. SF1 activates *Sox9* through a testis-specific enhancer element, termed *TES*. We tested the abilities of eleven clinical SF1 mutations to activate *TES* in reporter assays in HEK293T cells. Eight of the eleven SF1 mutants showed considerably reduced activation of TES compared to WT SF1. Furthermore, all mutations causing moderate to severe DSD phenotypes correlated with a more severe impairment of *TES* activation. In addition, all eleven of the mutants showed reduced synergistic activation of *TES* in co-transfection with the testis-determining co-factor SRY. Overall, this biochemical analysis of the function of mutant SF1 from DSD patients suggests that a failure of *SOX9* up regulation, due to reduced activation of *TES* during testis development, could be the primary cause of the DSD in some patients with SF1 mutations.

(1) Sekido and Lovell-Badge, Nature, 2008, 453(7197):930-4

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IDENTIFICATION AND CHARACTERISATION OF SURFACE PROTEIN COMPLEXES IN HUMAN SPERMATOZOA

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Upon leaving the testis mammalian spermatozoa are functionally incompetent and are thus unable to fertilize an oocyte. As the spermatozoa ascend the female reproductive tract, functional maturity is achieved through a complex cascade of biophysical and biochemical changes known as capacitation. An important aspect of this final maturation phase is the remodelling of the sperm surface architecture to enable it to interact with the zona pellucida, a glycoprotein matrix that surrounds the oocyte, and initiate fertilisation. While originally thought to be underpinned by a simple lock and key mechanism, emerging evidence has suggested that this interaction may instead be mediated by a multimeric recognition complex that is formed on the sperm surface during capacitation. However, to date the presence and composition of such a complex has yet to be described. Through the application of Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE), we have provided evidence that human spermatozoa express a number of high molecular weight protein complexes on their surface. Furthermore, the affinity of these surface expressed complexes for the zona pellucida was assessed utilising solubilised human zona pellucida and the technique of Far Western Blotting. Among the complexes that showed affinity for the zona pellucida we identified one comprising 14 subunits of the 20S proteasome. Interestingly, the 20S proteasome has previously been implicated in various aspects of mammalian fertilisation, including zona pellucida penetration and the acrosome reaction, although its precise role in these events has yet to be elucidated. Collectively, these results demonstrate the presence of multimeric protein complexes on the surface of human spermatozoa, and support their putative role in the initial interaction between the sperm and the zona pellucida. Our current research is focused on elucidation of the role of the 20S proteasome in human sperm-zona binding and further investigation of surface expressed protein complexes.

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SERTOLI CELL-SPECIFIC DISRUPTION OF THE ANDROGEN RECEPTOR DNA-BINDING DOMAIN REVEALS DIFFERENTIAL TEMPORAL CONTROL OF DISTINCT ANDROGEN-REGULATED GENES

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Androgen receptor (AR) actions are vital for spermatogenesis. However, in postnatal development male germ cells do not express AR, highlighting its key role in testicular somatic cells. We recently used a Cre-loxP strategy to determine the *in vivo* requirement of AR DNA-binding in Sertoli cell (SC) function. Transgenic (Tg) mice with Cre expression targeted by SC-specific *AMH* or *Abp* promoters were crossed with floxed-*Ar* (*Ar*^{flox}) mice for Cre-loxP inframe deletion of *Ar* exon 3, which encodes a zinc finger essential for the DNA-binding domain (DBD). SC-specific mutated AR$^{\Delta ex3}$(SCAR$^{\Delta ex3}$) produced infertile *AMH*.SCAR$^{\Delta ex3}$ and *Abp*.SCAR$^{\Delta ex3}$ males. Testes from adult homozygous TgCre$^{(+/+)}$ *AMH*.SCAR$^{\Delta ex3}$ or *Abp*.SCAR$^{\Delta ex3}$ males were 30% of normal size and exhibited meiotic arrest, whereas testes from hemizygous TgCre$^{(+/-)}$ *Abp*.SCAR$^{\Delta ex3}$ males were larger (47% normal) with more postmeiotic germ cell development. Despite marked Leydig cell hypertrophy, testicular expression of the adult Leydig marker *Hsd3b6* (RT-PCR) and normal intratesticular testosterone levels (LC-MS/MS) in SCAR$^{\Delta ex3}$ males indicated the presence of morphologically distinct but functional adult Leydig cells. SC-specific mutated AR $^{\Delta ex3}$ was predicted to disrupt classical AR-regulated pathways via loss of direct DNA interaction. Androgen-repressed testicular *Ngfr* expression (known to be via non-classical AR pathways) was not upregulated in SCAR$^{\Delta ex3}$ testes, suggesting maintenance of a non-classical mechanism independent of AR-DBD. In contrast, SC-specific *Rhox5* and *Eppin* transcription, regulated by divergent or classical androgen-response elements respectively, were both decreased in postnatal SCAR$^{\Delta ex3}$ *vs*. control testes, demonstrating SC-specific AR function as early as postnatal day 5. However, *Rhox5* expression declined dose-dependently, whereas *Eppin* expression increased, in adult TgCre$^{(+/-)}$ and TgCre$^{(+/+)}$ SCAR$^{\Delta ex3}$ testes, revealing differential temporal control for distinct AR-regulated transcripts. Thus, our SCAR$^{\Delta ex3}$ paradigm displayed dose-dependent TgCre-disruption of meiotic competence and post-meiotic development as well as gene expression, and represents a unique model to selectively differentiate AR-regulated genes.

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TGFB SIGNALING IN AN IN VITRO SEMINOMA MODEL

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Testicular cancer, the second most common malignancy in young men, has a 95% cure rate but can result in infertility or subfertility. Its incidence has increased significantly in recent decades(1). This cancer is thought to arise during embryogenesis, based on the persistence of embryonic germ cell markers such as Blimp1(2), Oct3/4(3) and Nanog(3) in adult seminoma cells. TCam2 cells are a recently characterised in vitro seminoma model(4). We show by Q-PCR and immunofluorescence that they also express these early germ cell markers. TGFβ signaling plays a key role during germ cell development, and is implicated in the development of testicular cancers(5,6). To investigate this further, we first determined whether the pathway is active in TCam2 cells. By Q-PCR we demonstrate expression of the TGFβ downstream transcription factors Smad 2, 3 and 4, and Activin type I and II receptors. Importantly, ActRIIA, which is undetectable in adult testicular germ cells, but readily detected in human foetal germ cells(7) and clinical seminoma samples(6), is readily detectable at both the mRNA and protein level in TCam2 cells. Furthermore, 24 hour treatment with Activin (5 and 50ng/ml) or BMP4 (5 and 50ng/ml) induces a 3-4 fold increase in ActRIIA mRNA levels, but not ActRIA, ActRIB or ActRIIB. Strikingly, in TCam2 cells BMP4 and to a lesser extent retinoic acid, but not activin, support survival and proliferation of TCam2 cells in the absence of serum. This is consistent with known roles of BMP4 and retinoic acid in enhancing murine foetal germ cell proliferation/self-renewal and survival(8,9), and activin inhibition of foetal murine germ cell proliferation(10).

This study is the first to demonstrate a functional response in seminoma cells consistent with their foetal germ cell-like identity and forms the basis for future mechanistic analyses of the role of TGFβ signaling in human testicular cancer.

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COMPARISON OF FASTING GLUCOSE WITH THE ORAL GLUCOSE TOLERANCE TEST TO SCREEN FOR DIABETES IN SUBJECTS RECEIVING CHRONIC LOW DOSE GLUCOCORTICOID THERAPY

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Glucocorticoid (GC) therapy may increase the prevalence of diabetes. Patients are usually screened for diabetes by measuring fasting glucose, with an oral glucose tolerance test (OGTT) recommended when fasting glucose is non-diagnostically elevated (5.6-6.9 mmol/L). The aim was to investigate whether fasting glucose can diagnose diabetes in subjects receiving long-term GC therapy. As GCs predominantly increase postprandial glucose, we hypothesized that fasting glucose would have poor sensitivity in this patient group.

Plasma glucose was measured before and 2 hrs after 75g glucose load in 60 subjects (37 women) who were not known to have diabetes and were receiving chronic (>6 months) prednisolone 4-10 mg/d for inflammatory arthritis or polymyalgia rheumatica. The diagnosis of diabetes was based on WHO criteria. HbA1c and clinical characteristics were recorded. The sensitivity and specificity for fasting glucose to diagnose diabetes was calculated. Receiver operator characteristic (ROC) curves were generated for fasting glucose and HbA1c.

The subjects mean age =70±10 yrs, BMI =28.9±5.9 kg/m^2, waist-hip ratio (WHR) =0.90±0.11 and prednisolone dose =6.5±2.1 mg/d. The prevalence of diabetes was 15% (9 of 60 subjects). However, fasting glucose was <7 mmol/L (4.2-6.6 mmol/L) in all diabetic subjects. Subjects with diabetes reported urinary frequency more often (7/9 vs 10/41, p=0.001) and tended to be older (75±10 vs 69±10 yrs, p=0.09), but did not differ significantly from non-diabetics in gender distribution, BMI, WHR, family history of diabetes or prednisolone dose. A fasting glucose ≥5.6 mmol/L had 86% specificity but only 33% sensitivity to diagnose diabetes. The area under the ROC curve was 0.71±0.09 for fasting glucose and 0.69±0.09 for HbA1c.

In summary, fasting glucose, HbA1c and clinical characteristics do not reliably predict the presence of diabetes in subjects on long-term GC therapy. We conclude that subjects receiving chronic GC therapy should be screened for diabetes using an OGTT.

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AN AUDIT OF GENETIC SCREENING PRACTICES FOR PHAEOCHROMOCYTOMA AND PARAGANGLIOMA

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Introduction: Recently germ-line mutations in apparently sporadic phaeochromocytomas and paragangliomas have been estimated at 5-25%.[1-4] In view of the clinical implications of these reports an audit of patients diagnosed with phaeochromocytomas and paragangliomas in South Eastern Sydney Area Health Service from 1999-2009 was performed.

Methods: Patients with a phaeochromocytoma or paraganglioma were identified through the anatomical pathology database. The data was matched with the hereditary cancer database to identify referred patients. Clinical data was accessed from medical records.

Results: 17 of 50 (34%) patients were referred for genetic assessment (11 phaeochromocytomas and 6 paragangliomas, - 4 retroperitoneal, one thoracic and one head and neck.) Referred patients were younger and more likely to have a positive family history. See Table 1

2 (2/17) had a clinical diagnosis of neurofibromatosis type 1; 2 were not tested for family reasons. 8/13 tested (62%) had a germ-line mutation (6/13 Von Hippel Lindau (VHL) mutations, 2/13 succinate dehydrogenase-B (SDHB)) 4/13 had no mutations identified, 1/13 results were pending. see Table 2.

In the non-referred group, 11 of 33 were identified with high risk features which using today's standards would warrant genetic assessment.

Conclusion: With such a high mutation detection rate (62%), it is likely our service is currently under-testing. What is the duty of care for the 11 patients identified with currently recognized high risk features? For the other 22 patients, the tension exists between resource allocation, the failed opportunity to prevent morbidity in mutation carriers and the potential harm caused by recontacting patients. This audit has raised the need for better education and defined protocol for testing in individuals with phaeochromocytoma and Paraganglioma.

Table 1.

	Referred for genetic testing	Not referred
Mean age (years)	30.1 (+-15.9)	53.2(+- 17.7)
Malignant disease (n)	1/17 (6%)	3/33 (9%)
Multifocal disease including bilateral phaeochromocytoma (n)	4/17(24%)	2/33(6%)
Family history (n)	6/17(35%)	2/33(6%)
Extra adrenal (abdominal) phaeochromocytoma/paraganglioma (n)	4/17(24%)	6/33(18%)

Table 2

Characteristics	VHL	SDHB	No Mutation found	Pending
No.	6	2	4	1
Male	4	1	2	1
Female	2	1	2	0
Mean age at diagnosis (years)	25.5	19	28	12
Age range (years)	6-45	14-24	11-40	12
Adrenal tumours (n)	6	1	2	0
Extra adrenal tumours (n)	0	1	2	1
Bilateral tumours (n)	3	0	0	0
Malignant tumours (n)	0	1	0	0

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SCREENING FOR ADRENOCORTICAL HYPERSECRETORY STATES IN TYPE 2 DIABETES MELLITUS: LOW FALSE-POSITIVE RATES WITH NOCTURNAL SALIVARY CORTISOL AND ALDOSTERONE-RENIN RATIO

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Objective: Subclinical Cushing's syndrome (SCS) and primary aldosteronism (PA) are associated with the metabolic syndrome (MS) and in type 2 diabetes mellitus (T2DM), have a reported prevalence of 0-9% and 14%, respectively.[1,2,3] SCS screening studies have used the 1mg overnight dexamethasone suppression test (DST), which has a high false-positive rate (> 30%). The optimal SCS screening protocol is not known, although nocturnal salivary cortisol (NSC) has excellent diagnostic accuracy for Cushing's syndrome and may be a better screening test for SCS. Our aims were to determine (1) the clinical utility of NSC in screening for SCS in selected T2DM outpatients (HbA_{1c} > 8%) and MS and (2) the prevalence of SCS and PA in this cohort.

Design: Clinical assessment for early Cushing's syndrome and biochemical evaluation for SCS and PA.

Methods: 106 participants (64 males) with T2DM were recruited from two tertiary referral centres. No-one had Cushing's syndrome. Screening was with NSC (SCS) and aldosterone-renin ratio (PA). Other measures included morning plasma ACTH, serum potassium and direct renin concentration. A subgroup (n=35) also had a 1mg DST.

Results: Three participants had an elevated NSC but further testing excluded SCS. No cases of PA were detected.

Conclusion: The NSC had a lower false-positive rate (3%) than previously reported for the 1mg DST. Given the excellent performance of NSC in detection of hypercortisolism, the low false-positive rate in SCS suggests it may be suitable for screening. However, screening for SCS should be selective, rather than routine, although optimum selection criteria are not yet established.

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PLASMA NITRATE/NITRITE (NOX) CORRELATED TO SEPSIS SEVERITY AND MORTALITY BUT NOT VASOPRESSOR REQUIREMENT

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Objectives. Nitric oxide concentrations are elevated in sepsis and their vasodilatory action may contribute to the development of hyperdynamic circulatory failure. Hydrocortisone infusion has been reported to reduce nitric oxide metabolite (NOx) concentrations and facilitate vasopressor withdrawal in septic shock. Our aim was to determine if NOx concentrations relate to (1) protocol-driven vasopressor initiation and withdrawal, and (2) plasma cortisol concentrations, from endogenous and exogenous sources. Demonstration of a relation

between NOx, cortisol and vasopressor requirement may provide an impetus towards the study of hydrocortisone mediated NOx suppression as a tool in sepsis management.

Design. A prospective study of sixty-two patients with severe sepsis admitted to the intensive care unit .

Measurements. Plasma NOx, total and free cortisol, and corticosteroid-binding globulin (CBG) concentrations were measured and related to protocol-driven vasopressor use for 7 days following admission.

Results. Patients who developed septic shock (*n*=35) had higher plasma NOx, total and free cortisol, and lower CBG concentrations than the non-septic shock group (*n*=27). Cortisol, CBG and NOx concentrations correlated with illness severity. Free cortisol, and to a lesser extent total cortisol, but not NOx concentrations, predicted septic shock. NOx concentrations were higher in non-survivors and the concentrations were characteristically stable within individuals but marked inter-individual differences were only partly accounted for by illness severity or renal dysfunction. NOx concentrations did not correlate with cortisol, did not relate to vasopressor requirement and were not suppressed by hydrocortisone treatment.

Conclusions. Nitric oxide production was increased in sepsis and related to survival, but not related to vasopressor requirement. Perhaps due to unexplained high interindividual variability NOx was not suppressed reproducibly by hydrocortisone. The results suggest that NOx may not be a suitable target for individualized hydrocortisone therapy.

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SHOULD TREATMENT FOR GLYCOCORTICOID-SUPPRESSIBLE HYPERALDOSTERONISM (GSH) BE COMMENCED LONG BEFORE HYPERTENSION DEVELOPS, AND, IF SO, WHICH?

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Introduction: Since GSH can be diagnosed at birth in known families using cord blood DNA, an important question is when to commence treatment in order to prevent unwanted cardiovascular changes due to aldosterone excess. Our studies in eight young normotensive affecteds reported five years ago revealed pulse wave velocities and echocardiography abnormalities compared with age and sex matched normal controls.

Objectives: To examine the consistency and possible progression of already described disturbances in cardiovascular structure and function in normotensive individuals with GSH.

Methods: Five of eight subjects with genetically proven GSH who had previously been shown to have structural and functional changes on echocardiography (compared with 24 age- and sex-matched normotensive controls) were restudied after 85±10.6 months of follow-up with measurement of office blood pressure (BP) and echocardiographic characteristics, including left ventricular (LV) wall thicknesses, parameters of LV diastolic filling and systolic function.

Results: Compared with the initial, previously reported evaluation, mean systolic BP remained similar (130.8±12.7 vs 128.2±15.1 mm Hg; p=0.5707) and diastolic BP increased (72.0±9.6 vs 85.2±9.3 mm Hg; p=0.0178). LV posterior wall (0.84±0.1 vs 1.01±0.1 cm; p=0.0282), LV mass (128.7±20.6 vs 193.2±59.5 g; p=0.034), LV mass index (72.2±9.7 vs 104.4±23.1 g/m^2; p=0.0269) and mitral inflow deceleration time (168.8±29.1 vs 207.0±35.6 ms; p=0.0266) increased after follow-up. There were no significant differences in LV diameters and volumes, interventricular septum, ejection fraction, CVIB, E/A wave ratio and E/E' ratio.

Conclusions: In GSH, aldosterone excess is associated with increased LV wall thicknesses, LV mass and reduced diastolic function, suggesting that specific treatment (either partial ACTH suppression or aldosterone blockade) should be commenced early and perhaps even long before hypertension develops. Given possible growth-retarding effects of glucocorticoids in children, and lack of perfect receptor specificity of spironolactone, the time has come to discuss optimal treatment for children with GSH.

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THE -344C/T POLYMORPHISM IN THE ALDOSTERONE SYNTHASE GENE (*CYP11B2*) AND LEFT VENTRICULAR REMODELLING FOLLOWING MYOCARDIAL INFARCTION

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Introduction: Aldosterone has important pathophysiological roles in hypertension and cardiovascular disease. It also has direct cardiac effects such as hypertrophy, fibrosis and perivascular inflammation. It is likely that aldosterone impacts on left ventricular remodelling (LVR) following myocardial infarction (MI). Aldosterone synthase is the enzyme which catalyses the final steps in aldosterone synthesis. A -344C/T polymorphism in the aldosterone synthase gene (*CYP11B2*) has been associated with hypertension and adverse outcome in heart failure. It was hypothesised that this genetic variant may affect LVR post-MI.

Methods: 93 patients who had suffered an acute MI had cardiac magnetic resonance imaging (cMRI) scans at baseline and 24 weeks. Plasma aldosterone was measured on admission and at 24 weeks by radioimmunoassay. The DNA of these patients was subsequently sequenced for the *CYP11B2* variant.

Results: Baseline characteristics were described. Left ventricular ejection fraction (LVEF) was higher in the TT group than the CC group at baseline ($p = 0.04$). At 24 weeks left ventricular end systolic volume index was significantly lower ($p = 0.018$) and LVEF was significantly higher in TT than CT or CC genotypes. Over 24 weeks LVEF increased in the TT ($p = 0.003$) and CT ($p = 0.004$) groups but not CC. No other significant differences in cMRI parameters were identified between genotypes at baseline, at 24 weeks or over time. Additionally, no significant differences in plasma aldosterone levels were observed between genotype groups.

Conclusions: Although this study was not powered for genetic analysis, some statistically significant differences were apparent for cMRI indices. It may be that the -344C allele is associated with more extensive remodelling post-MI.

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ELEVATED SERUM INTERLEUKIN 6 LEVELS IN NORMOTENSIVE INDIVIDUALS WITH FAMILIAL HYPERALDOSTERONISM TYPE 1

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Objective: Experimental and clinical evidence suggests that aldosterone (aldo) excess is associated with adverse cardiovascular (CV) sequelae independently of its effects on blood pressure (BP). Results from animal studies suggest involvement of inflammatory pathways. We have previously reported that normotensive individuals with genetically-proven familial hyperaldosteronism type I (glucocorticoid remediable aldosteronism, FH-I) have increased echocardiographically-measured left ventricular wall thicknesses and reduced diastolic function when compared with normotensive controls matched for age, sex and BP [1]. In the current study, we sought evidence in these same individuals of aldo-mediated CV inflammation by measuring blood markers of inflammation. Design and Methods: In eight normotensive FH-I subjects and 24 normotensive controls (3 per subject), we measured serum or plasma interleukin 6 (IL-6) by Siemens Immunolite 2000 assay, osteopontin (OPN) using a human osteopontin ELISA kit (Immuno-Biological Laboratories, Japan), and highly sensitive CRP (hs-CRP) by Beckman IMMAGE immunoassay. Results: The FH-I group was well matched with controls for age (mean 26±14 SD vs 26±12y; NS), sex (females 63% in each) and 24 hour ambulatory BP (SBP 121±10 vs 118±10 mmHg, NS; DBP 72±12 vs 70±5 mmHg, NS), but demonstrated higher serum aldosterone levels (20.3±15.1 vs 11.5±8.0 ng/dL; $P<0.05$) and aldo/renin ratios (79.2±116.6 vs 7.2±5.1; $P<0.01$), and lower plasma K+ levels (3.8±0.1 vs 4.1±0.3; $P<0.05$), as expected. FH-I subjects had a higher mean level of IL-6 than controls (4.63±3.82 vs 2.51±1.35 pg/mL; $P<0.05$). Trends towards higher OPN (736±378 vs 594±487 ng/mL) and hs-CRP (6.5±15.2 vs 1.3±1.5 mg/L) levels did not reach statistical significance. Conclusions: Elevated IL-6 levels in normotensive subjects with FH-I support the concept that aldosterone excess induces cardiovascular injury through non BP-dependent, inflammatory-mediated mechanisms. Longitudinal studies are required to determine whether this could result in clinically significant cardiovascular dysfunction, justifying intervention in normotensive individuals with FH-I before development of hypertension.

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A HIGH-THROUGHPUT MASS SPECTROMETRIC METHOD FOR ALDOSTERONE MEASUREMENT: A STEP TOWARDS IMPROVEMENT DIAGNOSIS OF PRIMARY ALDOSTERONISM

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Development of reliable aldosterone methods, to aid detection of disorders of aldosterone regulation such as primary aldosteronism (PAL), is urgently required. This study sought to analytically and clinically validate a high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) approach to aldosterone measurement in human plasma. Samples were prepared by protein precipitation and aldosterone measured using on-line LC-MS/MS with d_7-aldosterone as internal standard. The assay was used to examine whether our current radioimmunoassay (RIA: DPC Coat-a-Count) might have led to either over- or under-diagnosis of PAL by fludrocortisone suppression testing (FST). A normal range for LC-MS/MS aldosterone was established by analyzing blood collected midmorning from 97 normotensive seated subjects.

The method was linear over the analytical range of 2.5-200 ng/dL ($r^2>0.994$, n=14). Inter- and intra-day accuracy and imprecision for quality control samples (6.0, 40.0, 150.0 ng/dL) were 92.2-102.0% and <6.3 % respectively (n=5). The lower limit of quantification was 2.5 ng/dL (inter- and intra-day accuracy and imprecision 91.4-94.5% and <9.5%; n=5). No interferences were observed in plasma from Addison's disease patients (n=5). Comparison of collection tubes revealed similar aldosterone results. Results obtained by LC-MS/MS for 16 patients with PAL confirmed by RIA supported the diagnosis (day 4 FST upright 1000h aldosterone >6.0 ng/dL) in each. In three patients undergoing FST following unilateral adrenalectomy for aldosterone-producing adenoma, both RIA and LC-MS/MS gave day 4 upright PA concentrations <2.5 ng/dL, confirming biochemical cure of PAL. Comparison of LC-MS/MS with RIA gave an acceptable mean bias (2.86%) but wide range (-44.8 to 54.4%) of differences. LC-MS/MS aldosterone concentrations in normotensive subjects ranged

from <2.5-22.9 (mean 7.4 ± 4.7 SD) ng/dL. This method of aldosterone measurement, the first reported that uses online-LC/MSMS, is precise across the clinically relevant range, not influenced by collection tube type, and offers benefits of semi-automated sample preparation and high throughput.

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EXPRESSION OF SMALL GLUTAMINE-RICH TETRATRICOPEPTIDE REPEAT-CONTAINING PROTEIN ALPHA (SGTA) IN RELATION TO ANDROGEN RECEPTOR EXPRESSION AND SUBCELLULAR LOCALISATION IN HUMAN OVARIAN TISSUES AND OVARIAN CANCER CELL LINES

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SGTA is an androgen receptor (AR) co-chaperone protein expressed in the epithelia of male organs that modulates AR activity by promoting cytoplasmic retention of the receptor. The ovary produces androgen hormones and many ovarian cell types express the AR. We hypothesize that SGTA is expressed in epithelial-derived cells of the ovary and can regulate ovarian cellular responses to androgen hormones. Expression of AR and SGTA was analyzed by immunohistochemistry in human ovaries (n = 8), fallopian tube (n = 2) and ovarian carcinomas (n = 25). We also examined AR and SGTA expression and ligand-stimulated AR nuclear translocation in three ovarian cancer cell lines: KGN, OVCAR3, SKOV3. Co-expression of AR and SGTA proteins was observed in all ovarian structures that give rise to ovarian cancers: the ovarian surface epithelium and the linings of the fallopian tube, inclusion cysts, and follicles. Surprisingly, SGTA was also strongly expressed in thecal cells, which are mesenchymal in origin. Cytoplasmic AR immunoreactivity was most evident in thecal cells, consistent with the proposed function of SGTA. In ovarian tumour cells, levels of AR and SGTA varied considerably but the two proteins had a highly significant positive correlation (p= 0.001). Compared to a prostate cancer cell line, C42B, AR expression was relatively low and SGTA expression relatively high in ovarian cancer cell lines. The AR:SGTA ratios were C42B (1.80) > KGN (0.55) > OVCAR3 (0.36) > SKOV3 (0.03) and the ability of dihydrotestosterone (DHT, 10nM) to induce nuclear translocation of AR, expressed as % positive nuclei following treatment, mirrored the AR:SGTA ratio: KGN (>90%), OVCAR3 (20%), SKOV3 (10%). Collectively, these results provide strong evidence for a biological relationship between AR and SGTA in ovarian tissues and form the basis for further investigation of AR signalling and its modulation by SGTA in normal and abnormal ovarian tissues.

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DEVELOPMENT AND VALIDATION OF A SENSITIVE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY ASSAY TO SIMULTANEOUSLY QUANTIFY ANDROGENS AND ESTROGENS FROM SERUM

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Immunoassays have been used for decades to quantify steroid hormones from biological samples. However, they are not ideal due to their lack of specificity, especially at low levels. Bench-top LC-MS/MS systems are now able to match the sensitivity of steroid immunoassays while preserving the reference level specificity together with capability of quantifying multiple analytes within a single run. This study aimed to develop a sensitive LC tandem MS method to simultaneously quantify important circulating androgens and estrogens from serum without derivatization in a single run and to apply this in a high-throughput setting.

A stable-isotope dilution LC-MS/MS method (using an API 5000 instrument) was established using atmospheric pressure photoionization (APPI) to simultaneously quantify testosterone (T), dihydrotestosterone (DHT), 17β-estradiol (E_2) and estrone (E_1) from serum (200 µL). The run was divided into two periods to allow estrogen detection in the negative ionization mode and then switching to androgen detection in the positive mode. Sample preparation involved liquid-liquid extraction with 1 mL hexane:ethyl acetate (3:2 ratio) containing deuterated internal standards. Calibrants and quality control samples (3 levels) were prepared in 4% BSA. Accuracy was assessed by spiked recovery of serum pools, and imprecision was assessed using the quality control samples.

Using 200 µL serum, limits of quantification were 2.5 pg/mL E_1 (9 pM), 5 pg/mL E_2 (18 pM), 10 pg/mL T (35 pM) and 50 pg/mL DHT (172 pM). Accuracy (93-110%) and precision (<15% at all levels) were within acceptable limits for bioanalytical method validation. An analysis time of 9 minutes allowed up to 150 samples to be processed per day. The method is sufficiently sensitive to quantify serum T in females and E_2 in males, which remain difficult to accurately quantify by immunoassay. It is also easily adapted to quantify steroid in tissue homogenates and in non-human samples (ie mouse).

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IMPACT OF NEONATAL TESTOSTERONE EXPOSURE ON THE PHYSIOLOGY AND STRESS INDUCED CORTISOL SECRETION OF GONADECTOMIZED PIGLETS

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Testosterone (T) influences the secretion of key metabolic hormones of the hypothalamic-pituitary (HP) axis. Neonatal manipulation has been shown to reprogram the sensitivity of the regulatory pathways for both growth hormone in rats (Jansson et al. 1987) and cortisol in pigs (Gallagher et al. 1999). In this study we initially investigated the acute physiological effects of T treatment before exploring whether neonatal T treatment altered metabolic status and the impact of stress on the HP adrenal (HPA) axis chronically.

Restraint-induced cortisol, IGF-I and individual weight gains were measured in 110 piglets across nine treatments (n=12 or 13 per group). At <18hrs post-birth, neonates received testosterone propionate (TP) at either: 0, 0.08, 0.8, 8.0mg/kg body weight. On Day 2, five piglets/group were blood sampled for measuring insulin and glucose status. On Day 4 all piglets were castrated except one group which had received no TP. On Day 24, half of each castrated group received either oil or 8.0mg/kg TP. On Day 25, blood samples were taken i) within 30 seconds of capture (for basal cortisol and IGF-I), and ii) after restraining for 2.5 minutes, to activate the HPA axis. Data were analysed using ANOVA with means separated by l.s.d. (P<0.05).

Neonatal plasma glucose and insulin and average daily growth rate to Day 24 were significantly higher (P<0.05) in piglets injected neonatally with 8.0mg/kg TP compared to 0mg/kg. Increasing concentrations of neonatal TP tended to increase plasma IGF-I concentration, with a significant difference between the 0.8 and 8.0mg/kg TP treatments (P=0.04). Acute stimulation with TP on Day 24, had no effect (P>0.05) on basal or stress-induced cortisol secretion.

The results indicate that whilst there was no evidence for long-term programming of the HPA axis in neonatal piglets, neonatal T administration increased growth vigour in which IGF-I status may be implicated.

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SGTA AS A REGULATOR OF ANDROGEN RECEPTOR SIGNALLING IN PROSTATE CANCER

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Androgens are essential for the growth and development of both the normal prostate and prostate cancer (PCa). The cellular actions of androgens are mediated by the androgen receptor (AR), a potent nuclear transcription factor. In the cytoplasm, nascent AR peptides interacts with chaperone molecules necessary for correct protein folding and the acquisition of high-affinity androgen binding capacity. Upon ligand binding, the AR translocates to nucleus and interacts with specific DNA response elements leading to the transcriptional regulation of androgen target genes. Recently, we described the biological actions of the co-chaperone, SGTA, on AR activity in PCa cells. SGTA becomes associated with the AR in the final stages of cytoplasmic maturation. While we know this has profound effects on downstream AR signalling, the precise structural regions of SGTA that mediate those effects are unknown. SGTA is a 36KDa protein that consists of a central tetratricopeptide (TPR) domain and a glutamine-rich (QRD) carboxyl terminus, and is thought to form a homodimer. The aim of this study was to map the SGTA residues required for homodimerisation, and to identify the role and requirement of SGTA domains on AR activity. Native PAGE gels confirmed SGTA exists as both a monomer (~20%) and homodimer (~80%). Broad deletion mapping using a mammalian two-hybrid assay demonstrated that only the first 80 amino acids of SGTA are necessary for homodimerisation. Finally, we used transactivation assays combined with a multitude of native response elements to define the role of individual SGTA structural domains and homodimerisation on AR sensitivity, specificity and transcriptional output in response to ligand. This study has established the residues required for SGTA dimerisation, and the SGTA domains that influence AR activity. Significantly, SGTA regions could be directly targeted by small molecular compounds as a novel means of modulating AR/androgen action in diseases such as PCa.

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THE EFFECTS OF PARATHYROID HORMONE AND PARATHYROID HORMONE-RELATED PROTEIN ON WNT SIGNALLING AND OTHER SIGNALLING PATHWAYS IN OSTEOBLASTS

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The observation that Wnt signalling was critical in bone metabolism has been a major development in the area of bone biology, with evidence linking the Wnt and Parathyroid Hormone (PTH) pathways in osteoblasts. We determined the effects of PTH, N- and C-terminal

peptides and full length PTHrP on signalling pathways using osteoblast (UMR106.01) cells transfected with pathway-specific luciferase reporter constructs, including CRE (PTH1R signalling target), TCF/LEF (Wnt pathway target) and 6xOSE2 (osteoblast-specific cis-acting element). PTH 1-34, PTHrP 1-34 and PTHrP 1-141 stimulated TCF/LEF-, CRE- and 6xOSE2-luciferase production in a time and dose-dependent manner with maximal stimulation at 4 hours, 10-50nM, respectively. There was no significant difference between PTH1-34 and PTHrP 1-141 stimulation of these reporters over a dose range of 0.08-50nM. Pre-treatment of UMR 106.01 cells with the PKA inhibitor H89 blocked the PTH and PTHrP stimulation of the TCF-, CRE- and 6xOSE2-reporter constructs. However, inhibition of either the PKC or MAPK pathways did not affect PTH/PTHrP actions on TCF-, CRE- and 6xOSE2-reporter constructs, suggesting that the cAMP/PKA pathway was the predominant mediator of PTH effects in osteoblasts. Mid-segment and C-terminal PTHrP did not stimulate these signalling pathways in UMR 106.01 cells but treatment of UMR 106.01 cells with C-terminal PTHrP for 2-4 hours appeared to have inhibitory effects on 6xOSE2 luciferase activity. PTH or PTHrP did not stimulate NFAT, NFkB or AP-1 dependent-reporter constructs. In addition, three different TCF/LEF-reporter constructs, which differ in the minimal promoters driving expression of a luciferase gene or in the numbers of TCF target sequences, were used and their relative responsiveness to PTH/PTHrP were documented. PTH and N-terminal PTHrP have pleiotropic effects on osteoblasts including stimulation of TCF/LEF, CRE and OSE2. Future work will help to further our understanding of cross-talk between PTH/cAMP signalling and Wnt pathway signalling.

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BONE DENSITY AND BODY COMPOSITION IN SPINA BIFIDA

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Spina bifida represents a variety of congenital neural tube defects and affects approximately 1 in every 1000 pregnancies. Though diverse in its degree of sensory and motor involvement, chronic problems include impaired mobility, bladder and bowel dysfunction, and scoliosis. Low bone mineral density in both children and adults with spina bifida has also been noted but reports of bone mineral density and body composition in these patients are not common. We studied 47 spina bifida patients cross-sectionally using dual energy x ray densitometry to further define this problem.

Results: The cohort consisted of 10 paediatric (< 18 years, 5 F and 5 M, age 11.5±3.9) and 37 adults (23 F, 14 M, age 30.3±7.6).

	L1-L2 t score	L1-L2 z score	Fem Neck t score	UD radius t score
Children	-	n=9 -2.7±1.7	-	-
Adults	n=28 -0..51±1.38	-	n=26 -1.37±1.17	n=20 0.71± 2.35

	BMI	Skeletal Muscle Mass	LeanTissue Mass/Ht^2	% fat
Children	n =10 23.5±9.6	-	n = 9 5.89±3.2	n=9 35.6±18.8
Adults	n=37 30.3±6.0	n=15, 18.3±5.1	n=29 6.6±1.3	n=29 40.3±11.5

Conclusions: This study demonstrates that femoral neck bone density is reduced in adults with spina bifida, whilst BMI and body fat are increased in both adults and children. Both low bone density and body composition need to be considered in the assessment of spina bifida patients.

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ATYPICAL FEMORAL SHAFT FRACTURES IN A POST MENOPAUSAL WOMAN AFTER LONG TERM BISPHOSPHONATE THERAPY

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We report a case of atypical minimal trauma femoral shaft fractures in a 56 year old lady who had effective treatment for her postmenopausal osteoporosis with Alendronate.

Our patient suffered a minimal trauma ankle fracture in 1999 and was commenced on Alendronate therapy in 2001. In 2008 she sustained a spontaneous right femoral shaft fracture while standing up from a sitting position. Histology of the femoral fracture site was negative for malignancy. Secondary osteoporosis and osteomalacia was ruled out on subsequent evaluation. In 2005, 4 years after the commencement of the bisphosphonate therapy, she was investigated for right hip pain and was diagnosed with enthesitis on a bone scan. On retrospective review, the 2005 bone scan demonstrated bilateral mid femoral shaft stress fractures rather than enthesitis. The site of the femoral shaft fracture in 2008 corresponded to the previously demonstrated stress fractures in the 2005 bone scan. The current femoral shaft fracture showed features consistently described in literature which are *transverse sub-trochanteric fracture, lateral cortical thickening* with *medical cortical spike*. She also had radiological signs of contra-lateral femoral stress fracture as described in other case series. Current bone scan with SPECT images confirms these findings. While the bone turn over marker was suppressed, the bone mineral density had improved significantly from 2001 to 2008. Our patient is due to have a tetracycline labelled bone biopsy followed by treatment with Teriparatide.

The case findings are consistent with the emerging literature in this area. We suggest that long term bisphosphonate therapy needs to be reviewed in a patient who suffers new minimal trauma femoral shaft fractures despite improvement in BMD. Contra-lateral femur should also be assessed with X-ray and bone scan. Prodromal thigh pain seems to be a warning symptom and we recommend this also be investigated in a similar fashion.

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BONE MINERAL DENSITY IN INDIGENOUS AUSTRALIANS: EGFR STUDY PILOT RESULTS

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Most data on bone mineral density (BMD), bone turnover and osteoporotic fractures originate from Caucasian studies. Reported ethnic variations include lower BMD in Aboriginal than white Canadian women, associated with lower lean body mass. Differences in anthropometry and body build exist both between and within Indigenous and Non-Indigenous Australian populations.

We analysed pilot data obtained from the larger "eGFR study" (Accurate Assessment of Renal Function in Indigenous Australians: multi-centre study across 5 strata of health, diabetes status, renal function and body composition. BMD (AP spine, femur) and body composition were assessed by DXA (Norland XR-46) in participants from 1 site: Top End, NT [mean (95%CI) eGFR 74 (55-100) ml/min/1.73m^2]. Vitamin D levels, bone turnover markers and fracture history are pending.

Characteristics [mean (SD)]:

	Females (n=20)	Males (n=8)
Age (years)	48 (11)	48 (14)
Height (cm)	161 (6)	173 (8)
Weight (kg)	72 (12)	89 (10)
BMI (kg/m^2)	28.1(4.5)	29.8(3.3)
Waist Circumference (cm)	97 (14)	107 (9)
Lean tissue mass (kg)	41 (6)	62 (7)
BMD femoral neck (g/cm^2)	0.95 (0.16)	1.05 (0.16)
BMD total hip (g/cm^2)	0.98(0.15)	1.11(0.16)
BMD L2-4 spine (g/cm^2)	1.16 (0.19)	1.34 (0.21)
T-score femoral neck* (SD)	-0.32(0.14)	-0.34(1.3)
T-score total hip*	0.16(1.2)	-0.14(1.3)
T-score L2-4 spine*	-0.13(1.09)	0.52(1.09)

*T scores for females compared to Australian population, males to Norland reference.

In the women, univariate correlations of total hip and spine BMD were significant for lean tissue mass (P=0.002 hip, 0.02 spine) and age (P =0.05 hip, spine). Only lean tissue mass and age were independent predictors in multivariate analysis; 57% and 43% of variance of total hip and spine BMD respectively.

Some studies suggest that fat mass largely drives the body weight contribution to BMD in Caucasians, however these data suggest that lean tissue mass is a better predictor in Australian Aboriginal people.

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VITAMIN D STATUS IN THE THIRD TRIMESTER AND NEWBORNS IN A SOUTH AUSTRALIAN COHORT

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The revised Australian Perinatal Practice Guidelines suggest vitamin D screening during the first appointment in all pregnant women at risk of vitamin D insufficiency. Poor vitamin D status in pregnant women has been reported worldwide. Epidemiological studies have shown maternal vitamin D deficiency to be predictive of pregnancy complications as well as poor neonatal outcomes. The current study was performed to determine the correlation between maternal serum 25(OH)D with neonatal levels.

Methods: Capillary plasma 25(OH)D was analysed using an enzyme immunoassay (IDS - EIA) in 100 consecutive newborn screening samples received over a 3 month period. Ten neonatal samples randomly selected were also analysed using tandem mass spectrometry (LC-MSMS). Maternal plasma 25(OH)D level measured during the 3rd trimester were correlated with newborn levels using regression analysis.

Results: The mean maternal age was 30 years. Of the newborns, 53% were female and 77% were term deliveries. Pre-eclampsia and premature rupture of membranes were reported in 8% and 9% of the pregnancies respectively. Maternal plasma 25(OH)D was measured in 19% of mothers in the first trimester, 42% in the second trimester and 69% in the third trimester. Neonatal 25(OH)D was related to maternal third trimester levels measured by EIA ($r = 0.3$;$P = 0.02$). EIA and LC-MSMS concentrations for newborns correlated significantly over a range between 20 to 103 nmol/L by EIA [$r = 0.9$; EIA = 1.04(LCMS) + 10.1].

Conclusions: Placental transfer of 25(OH)D depends on the maternal circulating plasma 25(OH)D level at least during the third trimester in concordance with published data. Neonatal plasma 25(OH)D obtained by IDS EIA correlate well with the gold standard HPLC-MSMS.

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METABOLISM OF SUBSTRATES INCORPORATED INTO PHOSPHOLIPID VESICLES BY 25-HYDROXYVITAMIN D3 1A-HYDROXYLASE (CYP27B1)

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CYP27B1 is a mitochondrial cytochrome P450 associated with the inner-mitochondrial membrane of kidney and other tissues. It catalyzes the 1α-hydroxylation of 25-hydroxyvitamin D (25(OH)D) producing 1α,25-dihydroxyvitamin D, the hormonally active form of vitamin D. To further characterize CYP27B1, it was expressed in E. coli and purified. Substrates were incorporated into phospholipid vesicles which served as a model of the inner mitochondrial membrane. Greater than 97% of the 25-hydroxyvitamin D3 substrate associated with the vesicles. CYP27B1 (0.5 μM) was able to metabolize 95% of the 25(OH)D3 in the vesicle membrane within 2 min, indicating that the cytochrome can rapidly access essentially all of the 25(OH)D3 in both halves of the phospholipid bilayer. Bovine and mouse adrenodoxin were equally effective in supporting CYP27B1-catalyzed hydroxylation of vesicle-associated substrate. The kinetics of 1α-hydroxylation of different substrates were measured with substrate concentrations being expressed as mol/mol of phospholipid in the membrane. 25(OH)D3 and 25(OH)D2 underwent 1α-hydroxylation with similar kinetics, the catalytic rate constants (k_{cat}) were 41 and 48 mol/min/mol P450, respectively, while K_m values were 0.0059 and 0.0045 mol/mol phospholipid, respectively. CYP27B1 showed inhibition when substrate concentrations in the membrane were greater than 4 times K_m, more pronounced with 25(OH)D3 than 25(OH)D2. CYP27B1 also catalyzed 1α-hydroxylation of 24,25-dihydroxyvitamin D3 and 20-hydroxyvitamin D3, but with lower efficiency than for 25(OH)D3. This study shows that CYP27B1 can hydroxylate 25(OH)D2 and 25(OH)D3 associated with phospholipid membranes with the highest activity yet reported for the enzyme. The low K_m values are consistent with the enzyme being very active with the low concentrations of substrate likely to be found in the mitochondrial membrane in vivo. We also show that the enzyme has low activity at very high concentrations of 25(OH)D3, revealing that substrate inhibition may contribute to the regulation of the activity of this enzyme.

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THE ROLE OF THE MAMMARY STROMA IN PARITY-INDUCED BREAST CANCER PROTECTION

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An early full term pregnancy reduces lifetime breast cancer risk by up to 50% whereas a later pregnancy (>35 years old) can increase risk. The protection offered by parity is proposed to be mediated by changes in the gene expression of the mammary gland and/or changes to the mammary stem cell numbers, culminating in decreased transformation susceptibility. A decrease in stem cell numbers has recently been reported following limiting dilution transplants of mouse mammary glands from young parous mice.We asked if this effect on stem cell activity was mediated by changes in the gene expression profiles within the basal stem cell compartment or its niche. Young mice (5-6 weeks) were subject to one full cycle of pregnancy, lactation and 10 weeks involution. Mammary stroma and epithelial cell sub-popualtions were isolated from groups of parous or age match virgin mice and RNA applied to Illumina mouse Ref6 full genome gene expression arrays (n=4 arrays per cell type per group). Raw expression data was variance stabilized and spline normalised using the lumi package in the Bioconductor software, before SAM analysis was performed to determine specfic changes in gene expression. Analysis revealed subpopulation specific genes but only identified significant effects of parity in gene expression of mammary fibroblasts and ER-negative luminal epithelial cells (829 and 184 genes respectively). Of the genes identified in the parous stroma, Decorin and Dkk3 were most highly expressed. These factors play anti-oncogenic roles in breast cancer development. This implicates the stroma in parity-induced protection from breast cancer. Within the ER-negative population, parous mammary glands showed altered DNA repair, cell cycle control

genes and those involved in inflammatory control. These preliminary observations suggest that early pregnancy induces changes in the mammary stroma and ER negative epithelial cells which may mediate protection against breast cancer development and mammary stem cell activity.

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SYNERGISTIC INDUCTION OF APOPTOSIS IN PROSTATE CANCER CELLS BY COMBINATIONS OF AGENTS THAT SUPPRESS ANDROGEN RECEPTOR SIGNALLING

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Growth and survival of prostate cancer cells are initially dependent upon androgens, and androgen ablation therapy (AAT) is used to control tumour growth. Unfortunately, resistance to AAT inevitably occurs, and there is an urgent need for better strategies to treat advanced prostate cancer. The intracellular mediator of androgen action, the androgen receptor (AR), is a key mediator of prostate tumour growth following failure of AAT. This suggests that targeting the receptor itself in addition to the ligand may be a more effective strategy than conventional AAT to eliminate AR-dependent prostate cancer cells. The objective of this study was to develop a combinatorial treatment strategy that would target multiple different aspects of AR function, utilising agents already in clinical use. We found that pharmacologically low levels of the AR antagonist bicalutamide markedly sensitised LNCaP prostate cancer cells to growth inhibition and induction of apoptosis by low doses of histone deacetylase inhibitors (vorinostat, LBH589), or the Hsp90 inhibitor 17AAG, that alone had no effect on cell survival. Induction of apoptosis by these combinations was associated with inhibition of AR signalling (assessed by expression of prostate-specific antigen and FKBP51), and could be prevented by addition of supra-physiological concentrations of androgen. Furthermore, the low doses of these agents had no effect, either alone or in combination, on growth and viability of AR negative PC-3 prostate cancer cells. Collectively, our observations suggest that combining different clinically-available AR-targeting agents may more effectively block AR function in prostate cancer cells, the majority of which are AR-dependent at all stages of progression, and thereby result in enhanced growth suppression and cell death. Clinically, this approach has the potential to prevent selection for cells with enhanced AR signalling, more effectively eliminate AR-dependent cancer cells, and thereby improve overall survival for men with prostate cancer.

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CHANGES IN DNA METHYLATION AND EXPRESSION STATUS OF GSTP1 IS A MARKER OF TREATMENT RESPONSE TO EPIGENETIC THERAPY IN PROSTATE CANCER

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Epigenetic modifications are heritable and reversible biochemical changes of the chromatin structure. Unlike mutations that involve an alteration in the DNA sequence, epigenetic modifications are able to regulate gene expression via chromatin remodelling. The most studied epigenetic modification is DNA methylation, which is the addition of a methyl group to a cytosine paired with a guanine (CpG) and is catalysed by DNA methyltransferases (DNMTs). CpG islands, which are clusters of CpGs, are frequently found within gene promoter regions and DNA methylation of CpG islands is linked to gene repression. Numerous studies support the important role of DNA methylation in carcinogenesis and DNMT inhibitors (DNMTi) are being tested in clinical trials for solid tumours. We investigated the effect of two DNMTi on prostate cancer cell viability and the DNA methylation and protein expression status of GSTP1. GSTP1 is a gene that is hypermethylated in nearly 100% of human prostate cancer and may be a useful tool for determining DNMTi efficacy. LNCaP prostate cancer cells treated with the DNMTi 5-aza-2'-deoxycytidine (5-aza) demonstrated dose-dependent growth suppression (≥0.05μM) and induction of cell death (≥0.5μM). Demethylation of GSTP1 was observed at doses corresponding to significant cell growth suppression (≥0.05μM). However, re-expression of the GSTP1 protein was observed only at doses where cell death was induced (≥0.5μM). Treatment of LNCaPs with a more stable DNMTi Zebularine required much higher doses (50-1000μM) to elicit dose-dependent cell growth suppression. The less potent action of Zebularine in inducing cell death was also accompanied by a lack of GSTP1 protein re-expression. We have shown that DNA methylation and protein status of GSTP1 is an indicator of DNMTi efficacy. Currently we are investigating the potential of GSTP1 as a marker for DNMTi efficacy in an in vivo model of prostate cancer.

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ROLE OF CYTOSOLIC PHOSPHOLIPASEA2-A IN PROSTATE CANCER CELL PROLIFERATION

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To discover novel therapeutic targets for the treatment of advanced prostate cancer requires understanding the association of the target with the altered biochemical pathways that play crucial roles in proliferation of prostate cancer cells. Emerging evidences indicate that an

increase in dietary intake of omega-6 polyunsaturated fatty acid such as arachidonic acid (AA), increases markedly prostate cancer progression in animal models of human prostate cancer. We propose that a blockade of cytosolic phospholipase A2-α (cPLA2-α), an enzyme responsible for cleaving thus making the dietary AA available for biochemical reaction, could have a therapeutic effect in treating advanced prostate cancer (1-2). Blocking or knocking down cPLA2-α in prostate cancer cells (LNCaP) with a pharmacological (Efipladib) or gene silencing approach (siRNA) resulted in a decrease in cell number, which was not due to apoptosis as analysed by TUNEL rather a cell cycle arrest indicated by an increase in number of cells in G1/G0 phase and a reduction in S phase. These changes were paralleled by reduction in BrdU incorporation and Ki-67 expression. Analysis of cell cycle regulators revealed a reduction in cyclin D1 expression (3). As Akt and androgen receptor play critical roles in prostate cancer development, these two key components were examined. Treatment with cPLA2-α inhibitor lowered both total and phospho-Akt levels. Interestingly, Efipladib also caused a reduction in androgen receptor expression. Taken together, these results demonstrate that inhibition of $cPLA_2$-α exerts an inhibitory effect on prostate cancer cell proliferation. The mechanism underlying the effect of cPLA2-α on prostate cancer cell proliferation is likely via PI3K/Akt (3-5) and androgen receptor signaling pathways. We conclude that cPLA2-α plays crucial role in proliferation of prostate cancer cells and thus can be considered as a potential therapeutic target.

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CONSTITUTIVE NF-KB SIGNALLING MAY PLAY A ROLE IN THE MOLECULAR PATHOGENESIS OF GRANULOSA CELL TUMOURS OF THE OVARY

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Granulosa cell tumours (GCT) of the ovary represent ~5% of malignant ovarian tumours. Despite considerable knowledge regarding granulosa cell biology, little is known about the molecular changes that give rise to GCT. The transcription factor nuclear factor κB (NF-κB) regulates genes involved in immunity, inflammation, cell proliferation and cell survival. Consistent with this role, misregulation of NF-κB has recently been linked to many forms of cancer and previous work in our laboratory has shown that NF-κB signalling is constitutively active in two human GCT-derived cell lines, COV434 and KGN[1]. We aim to determine the molecular basis of the NF-κB constitutive activity by targeted pathway inhibition. When COV434 and KGN cells are transiently transfected with a NF-κB reporter construct, constitutive activity is blocked by a chemical inhibitor of IκBα, BAY11-7082[1], suggesting aberrant activation occurs upstream of this point in the pathway. To further dissect the basis of the constitutive activity we targeted several pathway components; IRAK-1/4, RIP, IKKβ and IκBα, with chemical inhibitors using NF-κB reporter activity and cell proliferation/viability/apoptosis end points. While the inhibitor of IRAK-1/4 was able to inhibit IL-1 induced NF-κB activation, it did not inhibit the constitutive activity. Similarly, RIP was found not to contribute to the constitutive activity. Two inhibitors of the IKKβ subunit, a component of the NF-κB activation complex, were not able to inhibit the constitutive activity, but surprisingly, nor IL-1 or TNFα induced activity in COV434 and KGN cells. They were, however, able to inhibit IL-1 and TNFα induced activity in a control cell line, in which NF-κB signalling is not constitutive. These findings suggest IKKβ or the IKK complex may be central to the constitutive activity. The IKKβ gene sequence was analysed in COV434, KGN and T47D cells. We identified a previously reported, rare polymorphism (G1754A) in the KGN cells however no mutations were found in any of the cell lines. This study has potential significance in understanding the molecular pathogenesis of GCT and may act as a mechanism to identify novel therapeutic target(s).

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TESTOSTERONE SUPPLEMENTATION DURING ANASTROZOLE THERAPY DOES NOT INCREASE PROLIFERATION IN POSTMENOPAUSAL HUMAN BREAST TUMOUR TISSUE

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Aromatase inhibitors (AI) are the first line adjuvant therapy for postmenopausal women with estrogen receptor (ER) positive breast cancer. Side effects of AI therapy, such as arthralgia, can cause significant discomfort leading to compliance issues. T supplementation has emerged as a potential treatment for AI-associated arthralgia and has generated favourable results in a phase II clinical trial (NCT00497458). However, T replacement in breast cancer had been contraindicated until the advent of powerful 3rd generation AIs, such as anastrozole, that are highly efficacious in blocking conversion of T to estrogen, thus reducing estrogen-induced proliferative effects[1]. We tested the effects of T supplementation during AI treatment on tumour growth. Fresh breast tumour samples collected from 17 postmenopausal women were cut into 3 mm^3 pieces, cultured for 24h on gelatine sponges submersed in steroid depleted media and treated with vehicle (control), T (5nM) and/or AI (25ng/ml). Tissues were stained with antibodies for ER, progesterone receptor (PR), androgen receptor (AR), and Ki67 (marker of cell proliferation).

All tumour tissues were positive for ER, AR and PR (except for1 tumour that lacked PR). As expected for primary tissues, percent Ki67 positivity (mean; range) in the control was highly variable (6.45; 1-43.2). Tissue responses to T (7.9; 0.1-46), AI (6.06; 1.4-35.3), and T+AI (4.97; 0.7-26.1) were not significantly different from control (Wilcoxon signed rank test). However, the combination of T+AI showed a trend towards reduced Ki67 positivity compared to AI alone (p=0.07). In two patients T significantly increased Ki67 positivity by 2-4 fold, and in both instances this stimulatory effect of T was reduced to or below control values by treatment with AI.

Our results suggest that T supplementation during adjuvant AI therapy does not compromise AI-mediated inhibition of breast tumour growth and that combined therapy with T and AI may further enhance tumour suppression.

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PERI-OPERATIVE ASSESSMENT OF THE HYPOTHALAMIC / PITUITARY / ADRENAL (HPA) AXIS IN PATIENTS WITH PITUITARY ADENOMAS- AN AUSTRALASIAN SURVEY

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Surgical treatment of pituitary tumours can cause adrenocorticotrophic hormone (ACTH) deficiency. Perioperative glucocorticoids are thus often prescribed to prevent potential adrenal crises. However, there is a lack of consensus regarding the appropriate perioperative glucocorticoid regimen for pituitary surgery. We surveyed Australasian Endocrinology units to assess variability in the investigation and management of the hypothalamic / pituitary / adrenal (HPA) axis in patients undergoing transsphenoidal hypophysectomy.

A questionnaire was sent to one senior Endocrinologist at 18 centres performing pituitary surgery in Australasia. Respondents were asked to describe their investigation and management of the HPA axis based on hypothetical case vignettes of a non-functioning macroadenoma and a GH-secreting microadenoma undergoing transsphenoidal hypophysectomy.

Responses have been received from 15 of 18 centres (83%). In a patient with a macroadenoma, 8/15 assess the HPA axis preoperatively by only measuring morning cortisol, whereas 7/15 request a short synacthen test if morning cortisol is indeterminate. Perioperative glucocorticoids are prescribed by 13/15 (87%) of respondents for a patient with macroadenoma and an intact HPA axis. For a microadenoma 10/15 (67%) prescribed glucocorticoids. The duration of inpatient glucocorticoid therapy ranged from one single dose at surgical induction to the entire hospital stay. Following resection of a macroadenoma 12/15 respondents measure morning cortisol while in hospital to determine whether patients need additional outpatient testing of the HPA axis. Three of 15 respondents perform outpatient testing in all patients. In patients considered at risk of ACTH deficiency post-discharge, 6/15 request an insulin tolerance test, 5/15 a short synacthen test, 2/15 a metyrapone test and 2/15 measure morning cortisol.

In summary, there is wide variability in investigation and management of the perioperative glucocorticoid requirements of patients undergoing pituitary surgery in Australasia. This may reflect a need for large well-designed studies to better define appropriate perioperative management for patients with pituitary tumours.

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EPIGENETIC REGULATION OF LOCAL ESTROGEN BIOSYNTHESIS IN HUMAN BREAST ADIPOSE FIBROBLAST CELLS

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Estrogen plays a significant role in the development and progression of breast cancer (BC). Cytochrome aromatase p450, encoded by the gene CYP19A1, is the key enzyme catalysing the synthesis of estrogens from androgens. In postmenopausal women, adipose tissue becomes the major site for estrogen production coinciding with an increase in CYP19A1 expression in breast adipose fibroblasts (BAFs). Therefore, understanding the mechanisms of CYP19A1 regulation in BC is critical for developing therapeutic measures. Previous studies of CYP19A1 regulation in BC have focused on hormonal regulation of transcription via upstream tissue- and promoter- specific regulatory regions. While this is clearly important, it is increasingly evident that epigenetic events, such as DNA methylation, are a common mechanism regulating gene expression during the progression of cancers. The aim of this study was to investigate whether CYP19A1 expression is under epigenetic regulation, determining if such mechanisms contribute to the tissue- and promoter- specific expression of CYP19A1 observed in BC.

CYP19A1 transcripts in cancer-free BAFs stimulated with cytokines and glucocorticoids (e.g. TNFα, dexamethasone) are derived from distal promoter I.4, whereas breast tumour derived paracrine factors (e.g. prostaglandin E2) induce a regulatory switch to proximal promoters I.3 and II. DNA isolated from BAFs maintained under these two conditions was treated with sodium bisulfite allowing for methylation sequence analysis of CpG dinucleotides. Methylation mapping revealed a stochastic hetrogeneous level of methylation among pI.4 (9 CpG sites) and pI.3/II (11 CpG sites). No correlation was observed between elevated pI.3/II transcription and promoter methylation. In contrast, hypomethylation at pI.4 was observed following induction, suggesting potential inverse correlation. BAFs treated with 5-aza-2'-deoxycytidine - a DNA methylation inhibitor - increased total CYP19A1 mRNA expression ~50 fold in a dose dependant manner. This activation was deemed to be mediated via pI.4 by upregulation of trans-activating factors.

This study uncovers a new layer of complexity in the regulation of aromatase where CYP19A1 is indirectly inhibited by methylation in BAFs. These findings translate to BC by determining the methylation state of CYP19A1 and upstream transactivating factors in clinical BC specimens, and correlate with clinicopathological parameters.

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A NEW METHOD FOR XENOGRAFTING PRIMARY HUMAN PROSTATE CANCER USING TISSUE RECOMBINATION

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Prostate cancer (PCa) research is hindered by the lack of relevant preclinical models, since primary localized (versus metastatic) PCa tissues are extremely difficult to grow in the laboratory. However such models are required to study transition to advanced PCa. Previous studies show prostatic epithelial growth is dependent upon stromal paracrine signalling, using inductive and instructive developmental stroma (DS) from mice or rats in tissue recombination with human prostatic epithelia. In this study we hypothesized that successful xenograft of localised PCa will occur with firstly inclusion of DS, but also by selecting for enriched populations of tumour cells by sorting patient epithelia into $\alpha2\beta1^{hi/lo}$ populations where benign progenitor basal cells are predominantly in the $\alpha2\beta1^{hi}$ fraction, which are suspected to antagonise tumour growth. Isolated PCa cells from various Gleason grades of organ confined disease were grafted sub-renally as unsorted or sorted $\alpha2\beta1^{hi/lo}$ cells, with or without rat DS into adult NOD-SCID male hosts. Results indicated successful graft survival of 6/6 patient tumours in the presence of DS, whilst 0/2 patient tumours survived when grafted alone. For each patient we generated ~5 individual inoculations grafted with DS and overall survival (regardless of cell population) was ~58%. When grouping survival of inoculations into basal cell enriched ($\alpha2\beta1^{hi}$), depleted ($\alpha2\beta1^{lo}$) or unsorted subpopulations, $\alpha2\beta1^{lo}$ cells had greater survival (62%) then unsorted (54%) or $\alpha2\beta1^{hi}$ (50%) recombinants. Xenografted tumours contained hallmarks of PCa, including few p63+ basal cells and high levels of PSA expression. We have shown combining DS with PCa cells from organ confined disease increases grafting success. Secondly, fractionation of epithelial cells into $\alpha2\beta1^{hi/lo}$ populations increases the efficiency of this model and suggests tumour initiating cells do not arise from the basal/progenitor population. This model has wide utility and could be utilized in the future for pre-clinical drug trials.

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MEDICAL MANAGEMENT OF PRIMARY HYPERPARATHYROIDISM: IS PBS FUNDING NECESSARY?

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Four cases of primary hyperparathyroidism and successful cinacalcet use are reported. The first three represent acute management to allow safe surgery. The last represents chronic management, where surgery is contraindicated or declined.

This is not a currently approved indication for the use of cinacalcet in Australia. One case was suspicious for, and proved to be, a parathyroid carcinoma. While hypercalcaemia due to parathyroid carcinoma is an approved indication for the use of cinacalcet, the patient required treatment prior to the diagnosis being established. A fourth case is presented, in which an older patient, who is fearful of surgery and prefers to be treated medically, was successfully treated with cinacalcet. This indication has the approval of the Therapeutic Goods Administration (TGA), but not the funding support of the Pharmaceutical Benefits Scheme (PBS). The cases suggest that a broader range of indications and funding support for the use of cinacalcet is required, specifically including the temporary stabilization of hypercalcaemia due to primary hyperparathyroidism of any cause and to treat the biochemical manifestations of primary hyperparathyroidism, where surgical treatment is not an option (including a patient's reasonable objection to surgery).

85 year F, Ca2+ 3.10 , A-on-C bronchiectasis.

50 yr M, Ca2+ 2.73, complex congenital cardiac disease, needing cardiac surgery.

61 year M, progressive fatigue, Ca2+ 3.44, bradycardia, remote hospital.

85 year F, staghorn calculus, Ca2+ 2.61, uCa2+/Cr 0.79 mmol/mol (0.06-0.45), IHD controlled medically

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A CASE OF LUNG ADENOCARCINOMA METASTATIC TO THE PITUITARY GLAND CAUSING CENTRAL DIABETES INSIPIDUS

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Metatstatic disease to the pituitary gland occurs uncommonly. The most common primary sites of malignancy are the breast and lung. The presence of metastases to the pituitary gland generally indicates widespread malignant disease and portends a poor prognosis.

A 51-year old man presented with worsening dyspnoea and generalised aches over six weeks. He had polyuria and polydipsia for four months. He was an ex-smoker. CT examination of his chest revealed a mass in the lower lobe of the left lung, a large pericardial efffusion and multiple sites of presumed malignant disease. Fluid cytology obtained at pericardiocentesis confirmed metastatic adenocarcinoma.

In hospital, he was polyuric with his urine output exceeding 5 Litres/day. His serum sodium level was 148mmol/L and his serum osmolality was 307mM/kg which were both elevated with inappropiately low urine osmolality at 192mM/kg. A water deprivation test confirmed the presence of diabetes insipidus. MRI of the pituitary gland demonstrated a thickened and abnormally enhancing pituitary stalk with possible posterior gland involvement.

His anterior pituitary hormone profile revealed hypogonadism (FSH <1 IU/L, LH <1 IU/L, total testosterone 1.2 nmol/L). He was euthyroid and his serum prolactin was normal. His morning serum cortisol was 301 nmol/L and his ACTH was 3.7 pmol/L. He received corticosteroids with palliative chemoradiotherapy and he was commenced on Desmopressin and testosterone patches with good symptomatic improvement. Subsequently, he developed recurrent episodes of pneumonia and died six months following the time of diagnosis.

Most metastases to the pituitary gland are asymptomatic. However, the most common clinical presentation which occurs is diabetes insipidus. Anterior pituitary hormone dysfunction is often under-recognised here owing to its non-specific symptoms which are commonly attributed to malignancy. Thus, a high index of suspicion is required to diagnose pituitary metastases.

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HYPOPHOSPHATEMIC OSTEOMALACIA: A CASE REPORT

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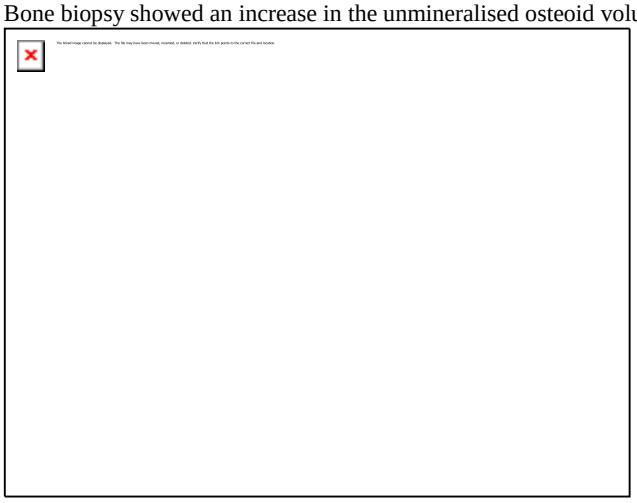
INTRODUCTION: Tumor induced osteomalacia (TIO) is a paraneoplastic syndrome characterised by hypophosphatemia, phosphaturia, inappropriately normal or low 1, 25 (OH)2 vitamin D concentration, myopathy and osteomalacia. We describe a case of suspected tumor induced osteomalacia with characteristic clinical and biochemical profile.

CASE REPORT: A 38 yr old lady presented with 10 months h/o severe bone pain involving the rib cage, back, shoulder and hip. She had waddling gait, proximal muscle weakness and tenderness over the rib cage, sternum, and spine.

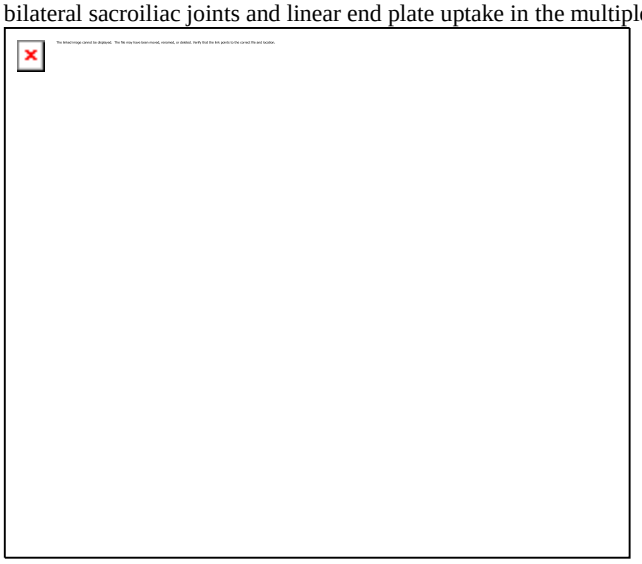
BIOCHEMICAL INVESTIGATIONS are shown in table 1.

Test	Baseline	After Tx
Total Ca	2.29mmol/L (2.10-2.55)	2.39
Phophate	0.40mmol/L (0.65-1.45)	0.78
ALP	238U/L (30-110)	
25 (OH) Vit D	124nmol/L (60-160)	
PTH	5.3 pmol/L (0.8-5.5)	8.8pmol/L
1,25(OH)2 Vit D	100 pmol/L (50-160)	165pmol/L
iFGF 23	61pg/ml (0-29)	
24hr urine PO4	22mmol/L	
24hr urine Ca	2.4mmol/L	
TmPGFR	0.4	
DEXA scan Neck of Femur	T-score -2.0 BMD 0.740g/cm2	

Bone biopsy showed an increase in the unmineralised osteoid volume with marked reduction in the trabecular volume. (Fig.1)



Whole Body Bone scan showed numerous foci of increased activity involving bilateral ribs, sternum, both humeral and femoral heads, bilateral sacroiliac joints and linear end plate uptake in the multiple vertebral bodies. Fig 2



CT of chest and abdomen and Indium 111 Octreotide scan was normal.

We treated her with Phospha soda and Calcitriol with significant improvement over two months and normalisation of serum phosphate.

DISCUSSION: Tumors responsible for oncogenic osteomalacia are usually benign rather than invasive and are often quite small and not detectable on physical examination or routine radiography (1). Tumors overproduce FGF-23 and this excessive amount cannot be adequately degraded by metalloprotenase a PHEX gene product (2). Successful localization of causative tumors with the use of indium-111 pentetreotide or octreotide scintigraphy has been demonstrated (3).

CONCLUSION: TIO is a clinical diagnosis in the absence of family history of XLH. Our patient's history, symptoms, signs and investigations are typical of TIO.

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MOTHER AND DAUGHTER WITH PITUITARY ADENOMAS: FAMILIAL OR CO-INCIDENTAL?

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Introduction: Aryl hydrocarbon receptor-interacting protein (*AIP)* mutations are reported in 15% of patients with familial isolated pituitary adenomas (FIPA)[1]. We present a FIPA family where no mutation was found and review the frequency of published *AIP* mutations.

Case: A 25 year old woman presented with typical symptoms and signs of acromegaly. Biochemistry confirmed elevated IGF-1 levels of 109nmol/L [RR: 14.2-36.9 age-adjusted], and failure of GH suppression following 75g glucose load. She had normal prolactin and other pituitary hormones levels. Pituitary MRI revealed a macroadenoma (17.3 x 21.5 x 18.4mm) with chiasmal compression. Transphenoidal resection resulted in symptomatic and biochemical resolution of her acromegaly. Histology confirmed a pituitary adenoma with positive immunostaining for GH and prolactin. Her 51 year old mother had presented 10 years earlier with secondary amenorrhoea,

hypogonadotrophic hypogonadism, hyperprolactinaemia, and elevated IGF-1 level. A 12mm pituitary macroadenoma was detected on MRI. She declined surgery and was treated medically with sandostatin LAR and cabergoline with symptomatic and biochemical improvement. Both patients have no clinical or biochemical features suggestive of *MEN 1* or Carney Complex. Genetic testing on *MEN 1* and *AIP* mutation were negative in index case.

Results: A total of 14 articles were found with *AIP* mutations in sporadic and/or familial pituitary adenomas published between 2006 (when the mutation was first discovered) and 2009. The majority of the studies were performed in European populations with only one study involving an Australian patient [2]. A substantial number of FIPA families do not harbour mutations in *AIP, MEN 1,* or *PRKAR1A* genes, suggesting a further as yet unidentified gene(s) may account for the remainder.

Conclusion: We propose a central data registry, especially among the Australasian families, to further characterise FIPA families in whom no mutation is found, to advance understanding of the biology of these familial tumours.

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HIGH PREVALENCE OF HYPONATRAEMIA AMONGST ACUTE MEDICAL ADMISSIONS IN TROPICAL NORTH QUEENSLAND

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Introduction: Hyponatraemia is known to be common in hospitalised patients. The factors associated with it are not well documented and there has been no major study in Australia.

Patients and Methods: We studied 11,205 admissions over two years (March 2007 – February 2009). The thresholds for mild, moderate, and severe hyponatraemia were respectively 2, 4 and 6 standard deviations from mean for non-hospitalised subjects.

Results: Admission sodium was available in 10,990 (98.1%). Hyponatraemia was present in 27.6% - mild 20.1%, moderate 5.2%, and severe 2.3%. There was no marked seasonal variation. Prevalence increased with age ($p < 0.001$), although mild hyponatraemia only increased up to age 50. Mild, moderate and severe hyponatraemia were present respectively in 19.6%, 4.5%, and 1.8% males and in 20.8%, 6.0%, and 2.8% females ($p < 0.001$). Mild, moderate and severe hyponatraemia were present respectively in 18.8%, 5.1%, and 2.4% of non-Indigenous patients compared with 30.0%, 6.5%, and 1.2% of Indigenous patients ($p < 0.001$). Those of mixed race were relatively protected. Hyponatraemia was present in 24.4% and 28.5% of non-Indigenous males and females respectively ($p < 0.001$) compared with 38.3% and 37.2% of Indigenous patients (not significant). Hyponatraemia was less common in cardiac (18.3%) and neurological (20.4%) patients compared with general medical (25.1%) while those with respiratory (27.4%), gastrointestinal (33.5%), infectious (35.8%), and metabolic (54.1%) conditions were predisposed. Prevalence in single admissions was 22.1% compared with 31.5% for patients who had a subsequent admission and 34.9% for readmissions (both $p < 0.001$).

Conclusions: Hyponatraemia is common amongst medical admissions in tropical Australia. The lack of seasonal variation was surprising. Separate reference ranges may be desirable for Indigenous and non-Indigenous subjects. Risk factors for hyponatraemia include advanced age, female gender (except in Indigenous subjects), and certain diagnostic categories. Low sodium is associated with increased risk of readmission.

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CASE REPORT: COMPLEX MANAGEMENT OF REFRACTORY HYPOGLYCAEMIA IN A MAN WITH MALIGNANT INSULINOMA

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A 64 year old man presented with symptoms of hypoglycaemia. A supervised prolonged fast induced symptomatic and biochemical hypoglycaemia. Plasma c-peptide, insulin and glucose levels demonstrated endogenous hyperinsulinaemia. A computed tomography scan identified lesions in the pancreatic tail and body, with multiple hepatic metastases. Endoscopic ultrasound and biopsy confirmed the diagnosis of malignant insulinoma. Hypoglycaemia persisted despite outpatient treatment with diazoxide, prednisolone and subcutaneous octreotide. Development of hypoglycaemic seizure required hospital admission and commencement of continuous intravenous 25% dextrose infusion. In view of inoperable status and demonstrated radiolabelled octreotide uptake on scintigraphy, the patient was treated with intravenous Lutetium-177 (Lu-177) labelled octreotate. Nevertheless, for the next forty days, the following inpatient therapies were still required for glycaemic support: intravenous dextrose, prednisolone, nocturnal nasogastric feeding and a continuous subcutaneous octreotide infusion (up to 3000mcg/day). Everolimus (5mg/day), an mTor inhibitor, was then commenced and this resulted in an immediate glucose response allowing withdrawal or significant reduction of the other supportive therapies. Complications potentially attributable to Everolimus therapy included fluid retention treated with spironolactone, localised herpes zoster skin infection and transient leukopaenia. The patient was successfully discharged from hospital after forty-six days with no further episodes of hypoglycaemia and improved insulin and c-peptide levels. Imaging 6 weeks post Lu-177 therapy showed reduction in size of hepatic metastases; this may be an effect of Lu-177 octreotate, everolimus or both. Capecitabine and temozolamide was commenced at discharge, with further regression in size of both hepatic and pancreatic lesions demonstrated after two chemotherapy cycles. Everolimus has been reduced with cessation planned.

SUMMARY: The management of refractory hypoglycaemia due to malignant insulinoma can be challenging and may need multiple therapeutic modalities. This case highlights the hyperglycaemic effectiveness of Everolimus in this setting. However, its role in tumour regression is uncertain.

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CUSHING'S SYNDROME IN AN IVF PREGNANCY

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Cushing's syndrome (CS) during pregnancy is a rare condition and may be overlooked due to overlapping features which include hypertension, fatigue and hyperglycaemia. Furthermore, there is increased hypothalamic-pituitary-adrenal axis activity and elevated corticosteroid binding globulin (CBG) in pregnancy leading to elevated total and free plasma cortisol levels and altered response to exogenous steroids, making the diagnosis more challenging. As CS results in increased maternal and fetal morbidity and mortality, early diagnosis and treatment are critical.

We report the case of a 32 year old female admitted to hospital 22 weeks pregnant with severe hypertension. She had previously been diagnosed with polycystic ovarian syndrome and underwent successful in vitro fertilisation. On admission, examination revealed a typical cushingoid appearance including moon facies, facial hirsuitism, acne, buffalo hump, truncal obesity and purple abdominal striae. She was also noted to be hyperglycaemic with glycosuria. Elevated 24-hour urinary free cortisol levels and suppressed ACTH were detected. An MRI at 24 weeks gestation revealed a right adrenal adenoma.

The patient underwent a laparoscopic right adrenalectomy at 26 weeks and received postoperative steroid replacement. Her hypertension and diabetes improved post-operatively, although she still required antihypertensive and insulin therapy throughout the remainder of her pregnancy. Her early morning cortisol levels remained detectable, possibly due to increased levels of placental hormones. She delivered a healthy infant at 36 weeks via caesarean section. Postpartum her basal cortisol levels suppressed completely, as expected. She developed postpartum depression.

In this case, undiagnosed CS complicated the patient's pregnancy and may have contributed to her inability to fall pregnant. This case illustrates the importance of comprehensive screening prior to assisted conception as well as the benefits of intervention in CS occurring in pregnancy.

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AN INVESTIGATION OF THE FIBRILLIN-3 GENE IN POLYCYSTIC OVARY SYNDROME AND EXPRESSION OF THE FIBRILLIN GENE FAMILY IN HUMAN OVARIAN TISSUE

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Several studies have demonstrated an association between polycystic ovary syndrome (PCOS) and the dinucleotide repeat microsatellite marker D19S884 which is located in intron 55 of the fibrillin-3 (*FBN3*) gene. Fibrillins, including FBN1 and 2, interact with latent transforming growth factor (TGF)- β binding proteins (LTBP) and thereby control the bioactivity of TGF β s. TGF β s are important for normal remodelling of ovarian tissue during the menstrual cycle via their ability to stimulate fibroblast replication and collagen production. The PCOS ovarian phenotype includes increased stromal collagen and expansion of the ovarian cortex, features possibly influenced by abnormal fibrillin expression.

To examine a possible role of fibrillins in PCOS, particularly FBN3, we undertook tagging analysis (32 SNPs including 10 that generate non-synonymous amino acid changes) using DNA from 173 PCOS patients and 194 controls. Alleles of most SNPs showed almost identical population frequencies between PCOS and control subjects and only one significant ($p < 0.05$) association was observed (rs3813774). Importantly, no significant differences were observed for the microsatellite D19S884. In human PCO stroma/cortex (n = 4) and non-PCO ovarian stroma (n = 9), follicles (n = 3) and corpora lutea (n = 3) and in human ovarian cancer cell lines (KGN, SKOV-3, OVCAR-5), *FBN1* mRNA levels were approximately 100 times greater than *FBN2* and 200-1000 fold greater than *FBN3*. Expression of *LTBP-1* mRNA was 3-fold greater than *LTBP-2*. We conclude that *FBN3* has little involvement in PCOS, but cannot rule out that other markers in the region of 19p13.2 are associated with PCOS . Alternatively, *FBN3* expression in organs other than the ovary may influence the PCOS phenotype.

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INSULIN-LIKE GROWTH FACTOR 1 AND ITS BINDING PROTEINS 1 AND 3 ARE DIFFERENTIALLY ASSOCIATED WITH METABOLIC SYNDROME IN OLDER MEN. THE HEALTH IN MEN STUDY

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Context: Levels of insulin-like growth factor 1 (IGF1) and its binding proteins have been variably associated with cardiovascular outcomes in men.

Objective: We sought to clarify relationships between IGF1 and IGF-binding proteins 1 and 3 (IGFBP1 and IGFBP3) and metabolic syndrome in older men.

Design, setting, participants: Cross-sectional analysis of 3,980 community-dwelling men aged ≥70 years.

Main outcome measures: Morning plasma levels of IGF1, IGFBP1 and IGFBP3 and presence of metabolic syndrome according to NCEP-ATPIII criteria.

Results: Increasing age was associated with lower plasma IGF1 and IGFBP3, but higher IGFBP1 (all $p<0.001$). Hypertension was associated with higher IGF1 ($p<0.001$) and IGFBP3 ($p=0.01$) and diabetes with lower IGFBP3 ($p<0.001$). For IGF1 and IGFBP3 there was a U-shaped relationship with middle quintiles possessing the lowest odds ratios (OR) for metabolic syndrome (reference: Q1 IGF1, OR for Q3 IGF1 0.74, 95% CI 0.57-0.96, reference: Q1 IGFBP3, OR for Q3 IGFBP3 0.67, 0.51-0.87). Increasing IGFBP1 was associated with reduced risk of metabolic syndrome with a dose-response gradient (reference: Q1 IGFBP1, OR for Q2 to Q5 0.56, 0.33, 0.22 and 0.12 respectively, $p<0.001$). Increasing IGF1/IGFBP1 ratio was associated with increasing OR for metabolic syndrome (Q1 to Q5, OR 1.00 to 5.42, $p<0.001$).

Conclusions: In older men, plasma IGF1 and its binding proteins 1 and 3 are differentially associated with metabolic syndrome. IGF1 and IGFBP3 levels in the middle of the distribution were associated with lowest risk, while increasing IGFBP1 was associated with reduced risk. Longitudinal follow-up of this cohort would determine whether distributions of IGF1, IGFBP1 and IGFBP3 predict incidence of cardiovascular events.

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IDENTIFICATION OF GROWTH HORMONE-REGULATED GENES IN ADIPOSE TISSUE: A MICROARRAY ANALYSIS IN HYPOPITUITARY MEN

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Adipose tissue is a major target of growth hormone (GH) action. GH stimulates lipolysis and reduces fat mass. The molecular basis underlying cellular and metabolic effects of GH in adipose tissue is not well understood. The aim of this project is to identify GH-responsive genes in adipose tissue.

Eight hypopituitary men underwent study of whole body energy metabolism and fat biopsies before and after one month of GH treatment 0.5mg daily. Total RNA was extracted using the RNeasy lipid tissue kit (Qiagen, Australia) and further purified by ethanol precipitation. Microarray analysis was undertaken using Agilent 44K G4112F arrays utilising a two-colour design. Differential expression was defined as an absolute fold change of ≥ 1.5 set at a false discovery rate of ≤ 15%.

GH increased circulating IGF-I and stimulated whole body fat oxidation. GH induced differential expression of 694 genes, 378 of which were up-regulated and 316 down-regulated. GH increased the expression of STAT3, SOCS3 and of PLCB2 (phospholipase C beta 2) that regulates calcium signalling. GH enhanced expression of IGF1 and FGF12. GH did not change the expression of key lipolytic enzymes, such as HSL (hormone-sensitive lipase) and ATGL (Desnutrin/adipose triglyceride lipase), but down-regulated PEPCK (phosphoenolpyruvate carboxykinase) and SCD5 (stearoyl CoA desaturase), enzymes involved in triacylglycerol synthesis. It repressed 11ß hydroxysteroid dehydrogenase, which stimulates the conversion of inactive cortisone to cortisol, a promoter of adipocyte differentiation. It increased the expression of PNPLA3 (adiponutrin), a novel protein marker of adipocyte differentiation and energy balance. In summary, GH regulates adipocyte genes encoding JAK/STAT and calcium signalling, growth, differentiation and those governing triglyceride synthesis. GH exerts differential transcriptional effects on adipocyte growth and differentiation, and on metabolism to reduce lipid storage.

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IN-VIVO CLEAVAGE OF HUMAN CORTICOSTEROID BINDING GLOBULIN (CBG) IN SEPSIS PATIENTS

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Human corticosteroid binding globulin (CBG) is a highly heterogeneous plasma steroid hormone binding protein that functions to regulate the bioavailability of the free biologically active hormone. CBG has also been shown to bind specific membrane receptors found on many target cells and post-receptor binding events include cAMP production, cortisol delivery and CBG/cortisol internalization. Based on the high sequence homology with the serine protease inhibitor alpha-1-antitrypsin and the functional interplay with neutrophil elastase at inflammatory sites CBG is shown to be a member of the Serine Proteinase Inhibitor (SERPIN) superfamily. Unlike the inhibitory SERPINS however, CBG has diverged throughout the course of evolution and is considered as a non-inhibitory steroid ligand binding SERPIN. As a SERPIN, CBG is responsible for the targeted delivery of the anti-inflammatory hormone, cortisol, at sites of inflammation. Neutrophil Elastase is known to cleave CBG towards the C terminus (at the so called reactive centre loop, RCL) disrupting the integrity of the steroid binding site leading to dumping of the 90% pool of steroid at sites where it is required. While purified CBG cleavage by PMN derived elastase can be demonstrated in-vitro, no cleavage of CBG has been seen in the serum of any individual. This study was undertaken to determine the presence of elastase cleavage products in the serum of sepsis patients. Results clearly show CBG is cleaved in sepsis but not in control serum . The degree of cleavage in-vivo differs to that in-vitro possibly due to other protective factors in-vivo which are absent in the in-vitro experiments. CBG cleavage in sepsis serum is associated with an over 50% reduction in binding capacity as expected. We conclude that in healthy serum (ie. non-inflammatory state) CBG remains uncleaved and in its stressed conformation and affinity for cortisol therefore is high. In sepsis serum (ie. systemic inflammatory state), findings show that CBG is cleaved to its relaxed conformation and has reduced affinity for cortisol. These results confirm that decreased steroid binding activity of CBG in sepsis patients is an outcome of CBG cleavage.

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PHOSPHORYLATION CONTRIBUTES TO HUMAN CORTICOSTEROID BINDING GLOBULIN (CBG) HETEROGENEITY

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Post-translational modifications (PTMs) regulate the synthesis and functions of human corticosteroid binding globulin (CBG), and thus far the only identified form of PTM is N-linked glycosylation. Human CBG purified from pooled serum of healthy individuals show heterogeneity (thought to result solely from N-linked glycosylation) when electrophoresed using both one- and two-dimensional gel electrophoresis (1 and 2D SDS-PAGE). PNGase-F treated human CBG shows a single band of molecular mass 43 kDa on 1D SDS-PAGE which is similar to the unprocessed predicted peptide mass (42, 646 Da). We assumed from this data that all the heterogeneity of human CBG is abolished. However, when PNGase-F treated CBG was further separated on 2D SDS-PAGE it appeared as 3 distinct spots with similar molecular masses. This indicated the possibility of additional PTMs. Therefore, we considered phosphorylation as a potential additional PTM on human CBG. PNGase-F treated human CBG was detected from a 2D gel using Phos-tag 540, a phosphoprotein gel stain and our data clearly shows that these 3 spots are reversibly phosphorylated. This is the first report showing phosphorylation, in addition to glycosylation, is a contributing factor to CBG heterogeneity.

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PERFORMANCE OF GLUCOSE METERS DURING HYPOGLYCAEMIC DYNAMIC TESTING

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Background: Insulin Tolerance Tests (ITT) and C-Peptide Suppression Tests (CPep-T) are dynamic blood tests which involve intravenous insulin therapy to cause hypoglycaemia with venous blood glucose levels (BGLs) maintained < below 2.5 mmol/L, while measurements are made for stimulated or suppressed metabolic hormones (cortisol, growth hormone and c-peptide). This requires close monitoring of BGLs at regular intervals with standard glucose meters since laboratory glucose values are not available to assist with decision making at bedside.

Aim: To establish the most reliable glucose monitoring systems in the hypoglycaemic range during ITT and CPep-T.

Method: Routine sequential whole blood samples were taken as per protocol at baseline and at regular intervals following the intravenous injection of a fast acting insulin. For this study these samples were tested in triplicate using 6 glucose meters- Accucheck "Performa", Accucheck "Advantage", Freestyle Lite, Optium Xceed 20s, True Track and one I-Stat Portable Clinical Analyzer . This was compared with glucose measurements on the laboratory multi channel hexokinase analyzer as reference method. The mean of the BGLs was used to calculate the absolute glucose difference in mmol/L and % bias was calculated as

(Meter glucose – Lab glucose) x 100

Lab glucose

Conclusion: When performing ITT's and CPep-Tests, BGL's need to be monitored by reliable and accurate machines that are comparable to the laboratory analysers. Some glucose monitors show significant disparity with these laboratory results whilst others reproduce results similar to the lab results. This study though small will show which devices are more accurate in the hypoglycaemic range.

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BASELINE SERUM CORTISOL IN PREDICTING ADRENAL SUFFICIENCY AS ASSESSED BY THE 250 MCG SYNACTHEN STIMULATION TESTS (SST) IN 625 PATIENTS

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Previous studies have demonstrated that a morning serum cortisol one week after pituitary surgery of <100 nmol/L makes further dynamic testing unnecessary to confirm adrenal insufficiency (AI). The morning cortisol level that reliably predicts adrenal sufficiency (AS) is more controversial and ranges from 400 to 500 nmol/L.

Aim: Determine the ambulatory morning cortisol level that predicts adrenal sufficiency using a Receiver Operating Characteristics (ROC) curve.

Method & Subjects: We conducted a retrospective audit of SST performed at PathWest Laboratory QEII from January 2006 to August 2008. A total of 764 results were obtained. Patients in intensive care and those on glucocorticoid therapy were excluded from the analysis (n=139). Baseline serum was obtained prior to intramuscular injection of 250 mcg Synacthen, with repeat venesection to gain the second sample 30 minutes post Synacthen. Cortisol was analysed on Immulite 2000 (Siemens). AS was defined as a 30 minute post Synacthen cortisol of >551 nmol/L; values <551 nmol/L were considered inadequate.

Results: Of the remaining 625 SST, 422 patients (68%) were AS whilst 203 (32%) were AI. ROC curve analysis indicated a basal cortisol cut-off of >236 nmol/L to predict AS (sensitivity 84%, specificity 72%). The positive predictive value (PPV) for AS if the basal cortisol value is >236 nmol/L is 85%, however 28% of cases within the AI group had a basal cortisol >236 nmol/L whereas only 3% had a basal cortisol >400 nmol/L .

Conclusion: A basal cortisol of >236 nmol/L is moderately reliable in predicting adrenal sufficiency, however to do this confidently a baseline cortisol of >400 nmol/L is required.

Table 1; Stratification of Patients by Basal Cortisol in Both Adrenal Insufficient & Adrenal Sufficient Groups.

Basal Cortisol Range (nmol/L)	Number in Adrenal Insufficient Group	Number in Adrenal Sufficient Group
< 100	57 (28.1%)	5 (1.2%)
101-200	65 (32.0%)	31 (7.4%)
201-300	47 (23.2%)	112 (26.5%)
301-400	28 (13.8%)	124 (29.4%)
401-500	6 (3%)	97 (22.9%)
> 500	0	53 (12.5%)
Total	203	422

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GLUCOCORTICOID RECEPTOR NULL MOUSE PLACENTAS ARE LARGER AND SHOW REDUCED MEAN HARMONIC THICKNESS RELATIVE TO WILDYPE LITTERMATES

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Glucocorticoids are essential for late fetal maturation but restrict placental and fetal growth. They act through the glucocorticoid receptor (GR), which is a ligand-dependent transcription modulator that belongs to the nuclear hormone receptor family. Homozygous deletion of the GR (GRKO) is lethal at birth due to respiratory failure. Previously we demonstrated that excess glucocorticoid exposure restricts fetal growth, decreases expression of placental Vegf and reduces placental vascularisation, but the role of the GR in placental development has not been fully defined. Therefore, we investigated the effects of GR deletion on the placental phenotype. GRKO heterozygous (+/-) mice were mated and on gestational day E18 (day 1 = E0) fetuses and placentas were collected, weighed and fetuses genotyped. Fetal weights were comparable in both genotypes whereas placental weights were increased by 13% in GRKO placentas (P=0.004, within litter paired t-test). Unbiased stereology was used to estimate volumes of junctional (JZ) and labyrinth (LZ) zones, and LZ component volumes (trophoblast (LT), maternal blood space (MBS) and fetal capillary (FC)), surface areas of MBS and FC and mean harmonic thickness (MHT). JZ and LZ volumes and LZ component volumes and surface areas did not differ between WT and GRKO placentas, but the MHT was reduced (21%; P=0.02) in GRKO placentas. Placental mRNA expression for peroxisome proliferator-activated receptor γ (PPARγ), 11β-HSD2, Vegfa, Igf2, Igfbp2 and the nutrient transporters SNAT-2, SNAT-4, GLUT-1 and GLUT-3, were determined by qRT-PCR. Expression of 11β-HSD2 was increased (46%; P<0.01) in the GRKO placentas whereas expression levels of all other genes were similar between genotypes. In conclusion, this study shows that absence of the GR increases placental weight, consistent with the known growth

inhibitory effects of glucocorticoids. Despite this, and an apparent increase in efficiency of the GRKO placenta (due to its reduced MHT), fetal weight remained unaffected.

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MANAGEMENT SURVEY OF ADDISON'S DISEASE DURING PREGNANCY: RELATION TO SELF-REPORTED PREGNANCY OUTCOMES

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Background: Pregnancy is associated with marked maternal cortisol elevations relative to the pre-partum state. There are few data to base recommendations for adjustments in glucocorticoid or mineralocorticoid therapy in women with Addison's disease (AD).

Objective: To survey current practice in management of AD during pregnancy in Australia, and retrospectively assess self-reported pregnancy outcomes. Methods: Volunteers, recruited through the Australian Addison's Association, completed a paper or web based questionnaire regarding their steroid management, symptoms and events during gestation, delivery and birth weight.

Results: Nineteen women (mean age 22.5 yrs) reported on 26 pregnancies. The average dose of hydrocortisone equivalent (mg) [HCTeq]/body surface area (m2) was 16.36mg/m2 (n=24). In approximately half of the pregnancies (14/26) patients were advised to increase their glucocorticoid dose, from a mean of 14.3 mg/m2 to 24.5 mg/m2 HCTeq. In 16/24 pregnancies women noted an increase in fatigue, nausea or dizziness, although the relationship of this symptom to altered steroid dosing could not be reliably determined in retrospect. In six pregnancies, women developed an Addisonian crisis requiring hospital admission and resuscitation, three of these had no increase in HCTeq dose during pregnancy. Only one of these six crisis patients had their HCTeq dose increased after the event. Average birth weight was 3.47kg (n=25), weight did not relate to glucocorticoid dose. Mean gestation was 38.7 weeks (R 35-42 weeks) Delivery was by caesarean section in 12/26 pregnancies and induction was utilised in 9/26 pregnancies. Only two pregnancies led to a normal vaginal delivery.

Conclusions: Current practice regarding glucocorticoid dosing in pregnant women with AD suggests clinical equipoise, resulting from a lack of data to guide clinicians. Our cohort reported a high frequency of self-reported Addisonian crises (6/26) and increased symptoms suggestive of glucocorticoid deficiency. High rates of assisted delivery were noted with good apparent foetal outcomes. Although this AD pregnancy cohort is large relative to current reports, larger retrospective datasets recruited by several means to reduce potential ascertainment bias, and objective verification, are required to confirm these outcomes. If confirmed, a prospective study to address optimum management of AD in pregnancy would be justified.

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SWITCHING FROM TWICE-DAILY EXENATIDE TO ONCE-DAILY LIRAGLUTIDE IMPROVES GLYCEMIC CONTROL IN T2D ON ORAL AGENTS

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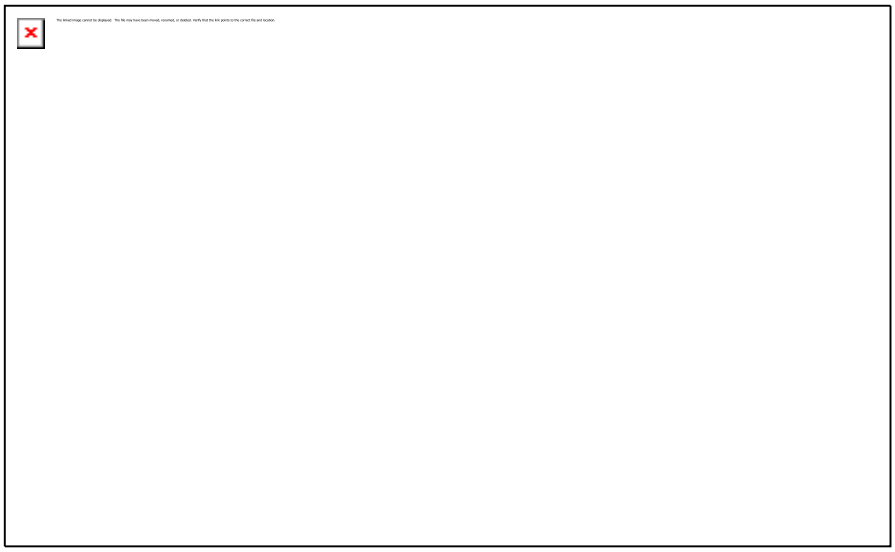
In a 26-wk randomised trial (LEAD-6), on a background of metformin and/or SU liraglutide was more effective than exenatide in improving HbA_{1C} and HOMA-B index of beta-cell function with less hypoglycaemia. In a 14-wk extension, subjects switched from exenatide 10µg BD to liraglutide 1.8mg/day, or continued liraglutide (100% [n=389] of completers participated in extension; 97% completed extension). Among participants switched from exenatide to liraglutide, improvements in various indices of glycemic control, as well as HOMA-B, bodyweight, and SBP occurred. The transition was characterised by a low incidence of minor hypoglycemia and nausea. Among participants continuing liraglutide, glycemic control was maintained with reductions in weight and SBP. One major hypoglycaemic event occurred with liraglutide. No pancreatitis occurred.

In conclusion, conversion from exenatide to liraglutide is well-tolerated and provides additional benefits in glycemic control and cardiometabolic risk factors.

Wk 0-26=ITT population in randomized trial. Wk 26-40=subjects who also entered 14-wk extension. Wk 26-40 comparison=Baseline was Wk 26 (LOCF) value. NS, non significant; NA, not applicable; †data are 0-40 wks. *p<0.05; **p≤0.001; ***p<0.0001.



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THE GENERATION OF A DOXYCYCLINE-INDUCIBLE, TISSUE-SPECIFIC AROMATASE TRANSGENIC MOUSE

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Aromatase catalyses the biosynthesis of estrogen and is widely expressed in the body. The aromatase knockout mouse (ArKO) becomes estrogen-deficient and develops unexpected phenotypes such as obesity and male-specific fatty liver and apoptosis at the hypothalamus. As circulating levels of estrogen in males are low, we hypothesize that local estrogen production in the brain may be important in regulating metabolic functions (e.g. liver lipid homeostasis) by acting in a paracrine or intracrine manner. To test this hypothesis, we are generating a brain-specific doxycycline-inducible, aromatase transgenic mouse.

The transgene (pTetOAROM) consists of the human aromatase cDNA (hAROM) and a *luciferase* marker placed under a bi-directional tetracycline-responsive promoter (pTetO), which is regulated by transactivators (rtTA or tTA) and doxycycline. This transgene increased hAROM transcription (16-fold, p=0.01), aromatase activity (3.4-folds, p=0.0008) and luciferase activity (16-fold, p=0.0006) in transfected MBA-MB-231tet cells that stably expresses rtTA, with doxycycline induction.

Pronuclear microinjection of the transgene produced four pTetOAROM founder mice (L1 to 4), which were bred with C57B6 WT mice to produce transgenic F1, with the following proportions of positive transgenics to total live births (ratios are of female to male positive pups): founder female L1 – 7/12 (4:3); male L2 - 7/26 (3:4); female L3 – 3/8 (2:1); male L4 - 9/31 (2:1).

A male pTetOAROM founder mouse was bred with a female mammary gland specific-rtTA mouse (MTB) to produce MTB-pTetOAROM double transgenic. Upon doxycycline treatment via drinking water, human aromatase expression was detected (by RT-PCR) specifically in mammary glands, salivary glands and seminal vesicles of double transgenic mice. Luciferase expression was also detected in these tissues by in vivo luciferase scan and in vitro luciferase assay.

In summary, we are generating a transgenic mouse model that expresses the human aromatase in a temporal- and spatial-specific manner.

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PRE-PROINSULIN SPECIFIC T CELLS CAN BE ISOLATED FROM THE ISLETS OF A T1D PANCREAS

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Histological studies of the pancreas in T1D have shown infiltration of islets with T cells. In addition, several studies provide evidence for the presence of autoantigen-specific T cells in the peripheral blood and pancreatic lymph nodes of T1D patients. Immune therapies aimed at preventing lymphocyte destruction of the beta cells require an understanding of the islet-infiltrating cells. However, data on islet infiltrating T cells from T1D subjects are scarce as pancreata from recent onset T1D are rarely available for study. We isolated islets from the pancreas

of a 19 year-old patient that was diagnosed 3 years prior with T1D. Flow cytometry performed on islets 12 hours post isolation showed increased expression of MHC class I HLA-A2 relative to non-diabetic islets. Lymphocytes were obtained from the T1D islets by culturing with IL-2 and IL-15 for 7 days. Flow cytometry revealed 38.8% of these cells were CD3+ T cells, of which 62.2% were CD4+ and 30% CD8+ T cells. Histology of the pancreas revealed CD8+ and CD4+ T cells infiltrating islets in situ. Recently, it was shown that processed epitopes from the human preproinsulin (PPI) signal peptide were major targets for circulating effector CD8+ T cells from HLA-A2+ patients with T1D. Using PPI15-24 HLA-A2 tetramer we demonstrated that <1% of the CD8+ T cells from the islets were specific for the PPI15-24 peptide. Five of these clones were stimulated with peptide and expanded using anti-CD3 antibody. This is the first study showing it is possible to culture lymphocytes of this specificity from the islets of a T1D pancreas.

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EFFECTS OF DIFFERENT LEVELS OF MATERNAL OVERNUTRITION ON OFFSPRING EARLY IN LIFE

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Maternal nutrition is a key determinant in programming fetal and newborn metabolism which may affect the risk of several chronic diseases including obesity and diabetes. We examined the effects of different degrees of maternal overnutrition on offspring early in life.

F0 founder female Sprague Dawley rats were fed chow (C) or high fat diet (HFD). F1 females from chow fed F0 were raised in normal (CN) or small (CS) litters (12 vs 3 pups/dam) and fed chow; a third group from HFD fed F0 was raised in normal litters and fed HFD (HN). Thus two degrees of overnutrition at F1 (CS, HN vs control CN) were implemented. Male and female F2 offspring from these F1 mother groups were culled at day 20 to collect brain and fat for mRNA expression. Plasma leptin, insulin and triglycerides were measured.

By 4 days of age, male and female HN offspring were heavier (vs CN offspring; $P < 0.01$). Male CS offspring were heavier from 7 days, and females from 13 day (vs CN offspring; $P < 0.05$). At day 20, only HN offspring were heavier in both genders with significant increases in plasma leptin and triglycerides (vs CN offspring; $P < 0.001$). Male and female HN offspring had 3 times more fat mass (vs CN offspring; $P < 0.001$). Maternal effects were not evident on insulin levels.

Our data suggest that the degree of obesity in F1 can program adiposity to F2 offspring early in life, with a greater impact of HFD induced maternal obesity than that induced by early overfeeding due to small litter size. The significant increase in CS offspring body weight during development suggests that modest increases in maternal body weight may play a small but important role in determining offspring metabolic risk.

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USE OF NOVEL BIOCHEMICAL AND TRADITIONAL CLINICAL RISK FACTORS TO ESTIMATE INSULIN RESISTANCE IN TYPE 1 DIABETES

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Insulin resistance is a feature of Type 1 diabetes and may contribute to the vascular complications associated with this disease. However, estimating insulin resistance in subjects with type 1 diabetes is limited due to the lack of simple clinical parameters that could reliably measure insulin resistance in this population. The clamp derived estimated glucose disposal rate (eGDR)[1], determined from routine clinical measures (hypertension, HbA1C and waist to hip ratio (WHR)) has been shown to account for approximately 60% of the glucose disposal rate in subjects with type 1 diabetes. Therefore, we sought to (1) validate this score in a separate cohort of 28 adult subjects (mean age 39.9 ± 12.9 years) with type 1 diabetes using euglycaemic-hyperinsulinaemic clamp studies, and (2) assess if the predictive value of the score could be improved further by the addition of novel biochemical [adipocytokines, hsCRP, cell adhesion molecules, oxidised LDL, advanced glycation end product (AGE), Asymmetric dimethyl Arginine (ADMA), nitric oxide] and clinical factors (body mass index (BMI), WHR, lipid profile, pulse wave analysis, blood pressure and tissue AGE) associated with insulin resistance. All subjects underwent a 4-hour euglycaemic-hyperinsulinaemic clamp ($60mU.m^{-2}$, min^{-1}). Glucose disposal rate (GDR) was determined during the last 60 minutes of the clamp. We found no correlation between the clamp determined GDR and the eGDR score in our cohort of subjects with type 1 diabetes (r^2=0.11, p=ns). Using linear regression, the combination of novel biochemical factors that yielded the highest adjusted r^2 value (0.51, p<0.002) were serum adiponectin, ICAM, and leptin; and BMI, diabetes duration and lens AGE were the combination of clinical factors that yielded the highest adjusted r^2 value (0.41, p<0.01). Thus, the addition of novel biochemical measures improves the ability to identify subjects with type 1 diabetes who are insulin resistant compared with simple clinical factors alone.

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EARLY LIFE OVERFEEDING LEADS TO OBESITY IN ADULTHOOD AND ALTERED NEUROIMMUNE RESPONSES TO LIPOPOLYSACCHARIDE

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Obesity is associated with detrimental effects on many aspects of physiology, including altered immune function that may partially underlie susceptibility to a variety of diseases. The extent of and mechanisms for this immune system dysfunction, however, have not yet been determined.

We have recently seen that rats made obese by overfeeding during the postnatal period have exacerbated paraventricular nucleus of the hypothalamus (PVN) responses to psychological stress, indicating a possible dysregulation of hypothalamic-pituitary-adrenal (HPA) axis function. We therefore hypothesized that obesity acquired during the animal's development would lead to immune system dysregulation and that this is associated with altered HPA axis function.

Litters of Wistar rats were reallocated at birth into small litters (4) or control litters (16). The rats were then examined, in adulthood, for febrile and HPA axis responses to an intraperitoneal immune challenge with the Gram-negative bacterial mimetic lipopolysaccharide (LPS; 100 µg/kg). Rats that were overfed as neonates (small litters) became obese in early life. Obesity was maintained through to adulthood and was associated with changes in metabolism. Importantly, rats overfed as neonates had exacerbated febrile responses to LPS, developing fevers that were approximately one degree higher than those from the control litters. These obese rats also had exacerbated PVN responses to the LPS, as demonstrated by an increased number of Fos-immunoreactive cells.

Overfeeding in early life has a remarkable long-term impact on physiology. Overfed neonates become obese and stay obese throughout adulthood. Here we present evidence that this neonatal overfeeding and the subsequent obesity may have serious detrimental effects on other aspects of physiology, including the ability to cope with an infection. We also suggest that an overactive HPA axis may be implicated in these effects.

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MITOCHONDRIAL MEDIATED APOPTOSIS BY CHRONIC EXPOSURE OF INS-1 RAT INSULIN CELLS TO HIGH LEVELS OF LINOLEIC ACID AND GLUCOSE

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Obesity with excessive level of free fatty acid (FFAs) in circulation is a factor linked tightly to type 2 diabetes. To gain insight into the mitochondrial regulated mechanisms by which elevated concentration of FFAs cause beta-cell dysfunction, we studied the long term effect of unsaturated FFAs, linoleic acid, on rat insulin cell line INS-1 cell apoptosis. Cells were incubated with 50, 250 or 500uM linoleic acid/0.5%(W/V) BSA for 48h under culture condition of 5, 11.1 or 25mM glucose in modified RPMI-1640 medium. Cell viability, apoptosis, gene expression, glucose stimulated insulin secretion(GSIS), mitochondrial membrane potential(MMP),mitochondrial permeability transition(MPT)opening and cytosolic Ca^{2+} concentration($[Ca^{2+}]i$) were measured.

Linoleic acid significantly increased cell proliferation at 50uM and 250uM, but remarkably suppressed cell viability and induced apoptosis at 500uM concentration in 5, 11.1 and 25mM glucose culture medium. compared to control and 24h treatment groups, we observed an increased in the expression of BAX, Bcl-2 and Bcl-2/BAX ratio after 48h treatment, suggesting the involvement of anti- and pro-apoptotic Bcl-2 family gene in FFAs induced apoptosis. It was found that the expression of another pro-apoptotic factor cytochrome c gradually elevated from 50uM to 500uM in cytosolic extracts but decreased in isolated mitochondrial compartment, which indicating a release of cytochrome c protein from mitochondrial to cytoplasm. Compared to mitochondrial uncoupler CCCP treated positivie control group, MMP of INS-1 cells showed significiantly dose-dependent attenuation by 50, 250 and 500uM linoleic acid treatment for 48h. With the increasing of glucose, linleic acid significantly suppressed GSIS and increased $[Ca^{2+}]i$.

These results suggest that, in INS-1 beta-cells, linoleic acid has a pattern of protection in low (50uM) to middle (250uM) concentration but have lipotoxicity in high (500uM) concentration by mitochondrial mediated apoptosis pathways, which involved increasing expression of pro-apoptotic gene, mitochondrial premeability transition opening, collapse of mitochondrial membrane potential and release the pro-apoptotic factor cytochrome c. Altough high concentration of glucose has protection effect compared with low glucose during lipotoxiciy, it still cause glucotoxicity by damage mitochondrial membrane potential.

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ROLE OF SEX HORMONES IN METABOLIC SYNDROME - LESSONS FROM THE AROMATASE KNOCKOUT (ARKO) MOUSE

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Metabolic syndrome is defined as a constellation of abnormalities associated with increased risk for the development of type II diabetes and cardiovascular disease, such as obesity, insulin resistance and dyslipidemia. Sex hormones including estrogens and androgens can influence the development and progression of these factors. To research the effects of sex hormones on metabolic syndrome, in particular insulin resistance, we studied Aromatase knockout (ArKO) mice which are unable to convert androgens to estrogens. Estrogen and testosterone are known to have bi-phasic effects on insulin resistance in a dose-dependent manner

Male and Female WT, ArKO and ArKO + E2 (17β-estradiol pellet, 0.0025mg/day) mice at 12, 24 and 52 wk-old were subject to Glucose, Insulin and Pyruvate Tolerance Tests after fasting. Sexually dimorphic differences were observed between the male and female ArKO mice. Male ArKO mice of all age groups present with signs of reduced insulin sensitivity which can be ameliorated by E2 treatment. Twelve wk-old female ArKO mice show signs of glucose intolerance with trends continuing at 24 wks also developing significant pyruvate intolerance and reduced insulin sensitivity. Surprisingly, E2 administration to 12 and 24 wk female ArKO mice, did not improve insulin sensitivity, instead, E2 induced further insulin resistance in 12 wk-old ArKO females. Further, E2 administration to 12wk WT mice induced a similar pattern of insulin resistance seen in the E2 treated female ArKO mice. By 52 wks, no differences were observed between the ArKO and WT females although. E2 administration to 52wk old female ArKO mice improved insulin sensitivity. Due to the differences in body weight, after E2 pelleting, 12 wk-old females have higher serum estrogen levels. Hence, our data indicates that high estrogen levels may induce insulin resistance. An example of a physiological state in which estrogen levels are high is pregnancy.

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PRADER-WILLI SYNDROME IS ASSOCIATED WITH ACTIVATION OF THE INNATE IMMUNE SYSTEM INDEPENDENTLY FROM CENTRAL ADIPOSITY AND INSULIN RESISTANCE

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Background: Prader-Willi syndrome (PWS) is the most common genetic cause of severe obesity. Despite PWS subjects having reduced visceral adiposity and less insulin resistance compared to obesity-matched non-PWS subjects, they still have reduced life expectancy due to cardiovascular comorbidities. Increased systemic low-grade inflammation has been described in PWS as a possible cause, but in earlier studies subjects were not matched for central adiposity, a major contributor to systemic inflammation. Therefore, we compared PWS and obese subjects matched for body composition to assess markers of systemic inflammation.

Methods: A cross-sectional cohort study comparing 10 PWS (BMI 35.6±2.8 kg/m^2), 12 matched obese (BMI 34.7±1.4 kg/m^2) and 10 healthy normal weight subjects (BMI 21.4±0.4 kg/m^2) using dual-X-ray absorptiometry for body composition, flow cytometry for quantification of activation marker expression on immune cells, and ELISA for measurement of CRP, total and high molecular weight-adiponectin, and IL-6. Insulin resistance was estimated by HOMA-IR.

Results: PWS and Obese were matched for BMI, percentage body fat and central abdominal fat mass. Insulin resistance and adiponectin levels were similar in all three groups. Nevertheless, PWS subjects showed significantly higher IL-6 (5.0±1.1 vs 2.5±0.4 pg/ml, p=0.02) with a trend towards higher CRP levels compared to obese (9.4±3.3 vs 4.0±1.0 ng/ml, n.s.). Similarly, PWS had significantly higher expression of CD66b (rMFI 15.8±2.1 vs 8.1±0.7, p=0.001) and CD11b (rMFI 11.9±2.8 vs 3.8±1.0, p=0.01) on neutrophils, reflecting an activated innate immune system. Immune cell activation markers correlated positively with central fat mass in lean and obese (r=0.49, p<0.05), but not in PWS.

Conclusions: PWS matched for central adiposity demonstrate a similar degree of insulin resistance, but higher levels of inflammation markers and activation of the innate immune system. The lack of correlation between inflammation and central adiposity suggests that activation of innate immunity may be a specific genetic feature of PWS, independent from metabolic abnormalities. Systemic inflammation might be a suitable pharmacological target to decrease cardiovascular risk in PWS.

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EVIDENCE FOR A RAPID EFFECT OF ESTRADIOL ON KISSPEPTIN CELLS IN THE EWE

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Reproduction is regulated by the interaction of neural and hormonal signals that converge on hypothalamic neurons responsible for the pulsatile secretion of gonadotropin releasing hormone (GnRH). Estradiol exerts feedback effects on brain cells which are relayed to GnRH

cells. How these feedback signals are generated is not clear. Kisspeptin is a neuropeptide produced in the arcuate nucleus and the preoptic area. In the arcuate nucleus, virtually all kisspeptin cells express estrogen receptor α and are strongly implicated in the relay of both negative and positive feedback. Kisspeptins are encoded by the *Kiss1* gene, its expression in females is regulated by stage of ovarian cycle, ovariectomy and sex steroid treatment in a manner consistent with sex steroid negative and positive feedback. We hypothesised that rapid signalling of estrogen involves changes in second messenger phosphorylation in these kisspeptin cells. Adult ovariectomised Corriedale ewes of similar weight (3/group) received 25 µg estradiol-17β (E17β) or vehicle and were killed 15 or 30 min later. The brains were perfused with 4% formaldehyde and processed for immunohistochemistry. Using coronal sections cut at 40 microns through the arcuate nucleus, the effect of E17β treatment on the phosphorylation of CREB and ERK-1/2 was determined using double-label immunohistochemistry. The cells were localised with a kisspeptin-10 antibody (566, Caraty) and co-localisation of pCREB and pERK was with Cell Signaling antibodies. E17β treatment significantly ($P < 0.05$) reduced the number of kisspeptin cells co-expressing pCREB in sections from animals sampled 15 and 30 minutes after E17β treatment whereas, pERK-1/2 expression in these cells was reduced only at 30 min. These data show that rapid estrogen signalling (within 15 min) occurs in kisspeptin cells. This effect could be a mechanism for negative feedback and time-delayed positive feedback effects that are relayed from kisspeptin cells to GnRH cells. Supported by CONACYT (project 93421).

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IS JUNK FOOD ADDICTIVE? THE ROLE OF THE MESOLIMBIC REWARD PATHWAY

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The consumption of high fat and sugar foods causes dopamine release in the mesolimbic reward pathway not dissimilar to that caused by drugs of abuse. Repeated activation of the reward pathway is associated with the development of addiction. This study will explore the hypothesis that overconsumption of junk food (JF) results in altered expression of neuropeptides in the mesolimbic reward system which may lead to JF dependence.

Adult male (n=10) and female (n=10) Wistar rats were given *ad libitum* access to either a variety of human junk foods (JJ) or standard rat chow. After 2 months, JF was removed from JJ rats and replaced with standard chow. Rat behaviour was monitored over the subsequent 72h, after which rats were killed and nucleus accumbens isolated and frozen. mRNA expression of tyrosine hydroxylase (TH), dopamine receptors (D1R, D2R) and dopamine active transporter (DAT) was determined by qRTPCR.

After junk food removal, both male and female JJ rats consumed significantly less chow than controls (Male: JJ, 1075+73kJ; C, 1645+31kJ;P<0.05; Female: JJ, 595+61kJ; C, 1175+21 kJ;P<0.01) and lost weight over the JF deprivation period (Male: JJ, -2.61+0.41%; C, 0.11+0.39%;P<0.05; Female: JJ, -3.89+0.74%; C, 0.83+0.93%;P<0.01). In males, but not females, TH mRNA expression was lower in JJ group (JJ, $3.62x10^{-4}+5.37x10^{-5}$; C, $1.14x10^{-3}+2.24x10^{-4}$,$P$<0.01). D1R mRNA expression, however, was lower in the JF females, but not JJ males (JJ, $3.68x10^{-2}+3.66x10^{-3}$; C, $6.58x10^{-2}+6.65x10^{-3}$,$P$<0.05). D2R and DAT levels were not different between control and JJ rats in males or females.

Behavioural changes in JJ rats after JF removal provide evidence that chronic JF intake was associated with the development of JF dependence. Lower TH and D1R mRNA expression in male and female JJ rats respectively suggests that components of the dopaminergic reward pathway are modulated in response to chronic JF intake and subsequent JF removal in a sex-specific manner.

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REDUCED RF-AMIDE RELATED PEPTIDE (RFRP) GENE EXPRESSION IN THE FOLLICULAR PHASE OF THE EWE ESTROUS CYCLE PERMITS INCREASED LH SECRETION FROM GONADOTROPES

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RFRP-3 is a mammalian neuropeptide which is encoded by the RFRP gene and reduces gonadotropin secretion. In primary pituitary cell cultures, RFRP-3 inhibits GnRH stimulated LH release[1] and blocks the synthesis of gonadotropin subunits[2]. To determine if RFRP-3 can exert an inhibitory effect *in vivo*, we injected (i.v.) 200µg RFRP-3 into hypothalamo-pituitary disconnected (HPD) ewes (n=6) in combination with a pulse of GnRH (100ng). RFRP-3 treatment reduced ($P< 0.05$) the amplitude of the GnRH-induced LH response (1.2±0.1vs. 0.8±0.1 ng/ml). We further hypothesized that reduced RFRP-3 gene expression and action of the peptide during the follicular phase of the estrous cycle is permissive for pulsatile LH secretion. During the breeding season , RFRP mRNA expression in the dorsomedial nucleus/paraventricular nucleus was quantified in Corriedale ewes by *in situ* hybridization. Expression was measured during the luteal and late-follicular phases of the cycle (n=5/group). The level of expression/cell was higher (P<0.01) in the luteal phase of the cycle than in the follicular phase. We then determined whether elevation of RFRP-3 levels in the follicular phase of the cycle would block pulsatile LH secretion. Mid-follicular phase ewes were given iv infusions of RFRP-3 (priming dose 1 mg, followed by continuous infusion 1mg/h for 2h) or vehicle (n=4/group). Blood samples were collected every 10 min for 7 h and treatments were administered between 3-5h of the sampling period. RFRP-3 treatment eliminated pulsatile LH secretion, but saline treatment was without effect . Mean LH levels were reduced (P<0.05) by RFRP-3 treatment (0.32±0.10 vs. 0.06±0.01 ng/ml). We propose reduced RFRP expression permits an increase in the pulsatile secretion of LH during the follicular phase of the estrous cycle. The effect of iv infusion is most likely the result of RFRP-3 action on the pituitary gland [1, 2], but an effect on GnRH secretion cannot be ruled out.

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(2) Sari et al (2008) ESA Abstract 359

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OUR EXPERIENCE OF PROLACTINOMAS IN MALES

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Our experience with Bromocriptine

70 male patients with prolactinoma were studied from 1992 – 2000. The mean age at presentation was 36 yrs (range 16 – 65 yrs). Headache was the most common complaint (76%) followed by visual deterioration (67%), erectile dysfunction (30%), gynaecomastia (21%), primary infertility (6%) and seizures (6%). Six patients presented with extra ocular nerve palsy.

Seven patients (10%) had grade-A tumors, 11 (16%) had grade-B, 14 (20%) had grade-C, 7 (10%) had grade-D and 31 (44%) had grade-E tumors.

Their serum prolactin levels ranged from 200 – 22,259 ng/ml.Ten patients (14%) had pan hypopituitarism and 12 (17%) had partial hypopituitarism.

Sixty nine patients were treated with bromocriptine. Forty nine patients underwent trans-sphenoidal pituitary surgery.The indications for surgery included 1) inadequate response to medical treatment 2) inability to tolerate medication and 3) inability to afford long term medication.

Eight patients received radiation therapy without surgery, while 17 patients received radiation therapy following surgery.

Twenty seven patients were available for long term follow-up. The mean duration of follow-up was 3 years (range 1-8 yrs). Normalization of serum prolactin was achieved in 36 patients (73%). Seven of these patients (19%) subsequently relapsed.

Our experience with Cabergoline

Thirteen patients with prolactinoma treated with cabergoline were followed up from 2003 – 2007. The mean age at presentation was 39 years (range 18 – 35 years). Headache was the most common symptoms. The mean duration of symptoms was 16.5 months (1 to 48 months). Nine patients presented with poor vision. Their serum prolactin levels ranged from 818 – 25.000 ng/ml.

MRI revealed grade A tumor in 1, grade B in 2, grade C in 2, grade D in 3 and grade E in 5 patients. All received Cabergoline. Two patients subsequently underwent surgery and one underwent radiation therapy. Seven patients were available for long term follow up.

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PHAEOCHROMOCYTOMA, NEUROFIBROMATOSIS & GIST: A RARE COMBINATION?

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Phaeochromocytomas have been identified in 0.1 to 5.7% of patients with Neurofibromatosis Type 1 (NF1), a complex disease associated with multiple neoplasms, including gastrointestinal stromal tumours (GIST). GISTs have been reported in up to 8.8% of NF1 patients. We report a case with the rare triad of phaeochromocytoma, NF1 and GIST.

A 39-year-old male with NF1 and long-standing history of untreated hypertension, anxiety and alcohol abuse, presented with right flank pain following a fall. He described intermittent episodes of palpitations and headaches attributed to his alcohol usage and not investigated further, despite a family history that was significant for both NF1 and phaeochromocytoma in his mother. An abdominal CT performed to investigate right flank pain revealed a large right adrenal mass and a mesenteric lesion. Serum and 24-hour urine catecholamines were markedly elevated.

Following α and β- adrenergic blockade, a laparoscopic right adrenalectomy was performed, together with a mini-laparotomy for small bowel exploration. A 3 cm pedunculated lesion was identified and excised from the small bowel. Histopathology was consistent with phaeochromocytoma of the right adrenal gland, with no vascular invasion or necrosis. The intestinal lesion was a GIST (strongly positive for CD117) with some features of malignancy.

The simultaneous occurrence of phaeochromocytoma with NF1 and GIST is extremely rare with only 8 reported cases in the literature, mostly incidental findings during surgery. This could be because GISTs are often asymptomatic, difficult to detect preoperatively and hence under-reported. This case illustrates the importance of investigating for the presence of phaeochromocytoma in patients with NF1 who present with hypertension. It is suggestive that patients with phaeochromocytomas and NF1 should have a trans-abdominal surgical approach to detect GISTs, a finding which would impact on management and prognosis.

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PERIPHERAL NEUROPEPTIDE Y Y1-RECEPTORS REGULATE LIPID OXIDATION AND FAT ACCRETION

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Neuropeptide Y (NPY) is known for its powerful effects on feeding, but its role in other critical aspects of energy homeostasis is largely unknown. Here we demonstrate that peripheral Y1 receptors play a major role in the control of substrate oxidation and lipid accretion. Germline Y1-/- mice of both genders exhibit increased physical activity and decreased respiratory exchange ratio, indicative of increased lipid oxidation. Although these mice develop late onset obesity, increased lipid oxidation is a primary effect of Y1 deletion rather than secondary to increased adiposity, as young lean Y1-/- mice show the same effect. The mechanism behind this is likely due to increased liver and muscle protein levels of carnitine palmitoyltransferase-1 (CPT-1) and maximal activity of key enzymes involved in β-oxidation; β-hydroxyacyl CoA dehydrogenase (βHAD) and medium-chain acyl-CoA dehydrogenase (MCAD), leading to increased mitochondrial capacity for fatty acid transport and oxidation. These effects are controlled by peripheral Y1 receptor signalling, since adult-onset conditional Y1 knockout in peripheral tissues also leads to increased lipid oxidation, liver CPT-1 levels and βHAD activity. Importantly, these mice are also resistant to diet-induced obesity. Together, this work demonstrates the primary role of peripheral Y1 receptors in the regulation of oxidative fuel selection and adiposity, opening up new avenues for anti-obesity treatments by targeting energy utilisation in peripheral tissues rather then suppressing appetite by central effects.

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DISRUPTION OF NUCLEAR STRUCTURE ALTERS PROGESTERONE SIGNALLING IN BREAST CELLS

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Progesterone, through its nuclear receptor (PR), plays a pivotal role in the development of the normal breast and is implicated in breast cancer biology. Ligand activation elicits PR dimerization, cofactor recruitment and binding to target genes to affect transcriptional regulation. We have shown previously that ligand activation causes the rapid movement of PR into subnuclear foci, which are sites of active transcription. We have also shown that tethering of PR to the nuclear matrix, the riboprotein scaffold responsible for the functional and physical compartmentalisation of the nucleus into discrete domains, is required for movement into foci and transcriptional activity. Movement of PR into foci differs in normal and malignant tissues: PR foci are larger in cancers, and ligand dependence is lost. We hypothesized that these differences in foci were due in part to the different structure of the nucleus in normal cells and cancers. In order to address this hypothesis, we perturbed nuclear structure in MCF-10A minimally transformed breast epithelial cells by ablation of the integral nuclear matrix protein SATB1. Impact on nuclear structure and PR target gene selection were determined by image analysis and gene expression profiling of progesterone-treated (100nM) MCF-10A cells expressing PR. Knock-down of SATB1 decreased nuclear size parameters and increased numbers of nucleoli, a feature common to cancer cells. Moreover, although PR regulated largely the same suite of genes after SATB1 ablation, there were gene sets that lost or gained sensitivity to regulation by PR. Functional pathway analysis identified enrichment in genes involved in transcription and proliferation after SATB1 ablation. Taken together, the results of this study demonstrate that nuclear structure is a critical component of the specificity of PR action in breast cells and delineation of the mechanisms involved may lead to further understanding of the role of progesterone signalling in breast cancer.

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PROTEIN ARGININE METHYLTRANSFERASE 6 (PRMT6) IS INVOLVED IN STEROID HORMONE RECEPTOR MEDIATED GENE EXPRESSION IN BREAST CANCER CELLS

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It is well established that transcription and splicing events are functionally coupled to regulate gene expression. Certain transcriptional cofactors control both of these processes and alterations in their levels can have profound effects on cellular functions. We have identified protein arginine methyltransferase 6 (PRMT6) as a regulator of both steroid hormone (SH)-dependent transcription and SH-independent alternative splicing. PRMT6 coactivates the estrogen (E2), progesterone and glucocorticoid receptors in luciferase reporter assays in a ligand-dependent manner. A mutant PRMT6 that is unable to methylate arginine residues on its target proteins is unable to function as a coactivator, demonstrating that PRMT6 requires its enzymatic activity to stimulate transcription. In order to investigate the importance of PRMT6 in the transcription of endogenous genes, we determined the effects of siRNA knockdown of PRMT6 on the expression of E2-inducible genes in MCF-7 cells. Taqman real-time PCR analysis revealed that the expression of GREB1 was reduced by 40% and progesterone receptor by 38% compared to control siRNA, demonstrating that PRMT6 is an integral component of the E2 signaling pathway. In contrast to its effects on transcription, the regulation of alternative splicing by PRMT6 is hormone-independent. Knockdown of PRMT6 in MCF-7 cells significantly increases the exon inclusion:skipping ratio of the endogenous VEGF and Syk genes in both the presence and absence of E2. These results demonstrate that PRMT6 has a dual role in regulating gene expression and these processes can occur independently of each other.

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ROLE OF GLUCOCORTICOIDS IN THE REGULATION OF ADIPOGENESIS

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Obesity is a serious health problem in the Western world. Important steroid hormones that are proposed to mediate adipose development in obesity and maintain metabolic homeostasis are the glucocorticoids. Distinct changes in the adiposity of patients receiving glucocorticoid therapy and in Cushingoid patients indicate that glucocorticoids play a critical role in regulating adipose tissue development and function. To investigate the molecular relationship between glucocorticoid action and adipogenesis, this study utilized a mouse model lacking an essential mediator for glucocorticoid signalling, the glucocorticoid receptor (GR).

Mouse embryonic fibroblasts derived from fetal GR-deficient and wildtype (WT) mice were cultured over a two week period and differentiated into a fat cell lineage. The resulting numbers of fat cells were compared by quantification with Oil Red O method. Preliminary results indicate that fat cells isolated from GR-deficient mice contained significantly lower lipid levels and total fat cell numbers when compared to the WT mice. Gene expression analysis was completed using quantitative RT-PCR on several markers of adipocyte development (C/EBPα , Glut 4, IRS-1, leptin, TNFα and PPARγ). In summary, these findings indicate glucocorticoid signalling via GR is an important pathway leading to adipocyte differentiation and may provide a potential target for therapeutics against obesity.

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MOLECULAR MECHANISMS CONTROLLING PROSTAGLANDIN ENDOPEROXIDE SYNTHASE-2 (PTGS2) GENE ACTIVITY BY GLUCOCORTICOID IN TERM AMNION

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Prostaglandins generated by PTGS2 in the amnion are critical for labour. We reported previously that PTGS2 mRNA expression and gene activity increased spontaneously in short term (24h) amnion explants[1] and was reduced by glucocorticoid[2]. Furthermore, glucocorticoid receptor (GR) was bound to the PTGS2 promoter in term amnion[2] suggesting chronic glucocorticoid-inhibition of PTGS2 expression in vivo. Here we explored the molecular mechanisms of the glucocorticoid control of PTGS2 gene activity in term amnion.

Amnion tissue (n=4) was incubated in serum-free media for 0, 4 and 24 hours with and without dexamethasone (DEX;100nM), and GR and RNA-polymerase-II (pol-II) binding, histone (H3 and H4) acetylation and H3K4 di-methylation were determined at the PTGS2 gene by chromatin immunoprecipitation.

GR binding to the PTGS2 promoter fell precipitously by 4h and rebounded partially by 24h, in agreement with gene activity changes. DEX blocked the fall at 4h, but not at 24h. Pol-II distribution (total, Ser-5 and Ser-2-phosphorylated) along the PTGS2 gene indicated polymerase stalling at exon-1 with a minority of pol-II engaged in elongation. Stalled initiator pol-II declined by 4h and was not preserved by DEX. Downstream binding of elongator (Ser-2-phosphorylated) pol-II was more stable, and elongator: initiator pol-II ratio slightly increased in culture and by DEX. H3 and H4 acetylation decreased by 50% and H3K4 di-methylation did not change at the PTGS2 promoter in culture , and these patterns were unaffected by DEX. Thus, the PTGS2 gene in amnion is controlled by transcriptional elongation, and GR may bind and function at the productively elongating genes even in the absence of steroid. The robust down-regulation PTGS2 mRNA levels by DEX may involve a separate, possibly post-transcriptional mechanism, which remains to be established.

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HUMAN PLACENTAL LACTOGEN REGULATES PREGNANCY INDUCED INSULIN RESISTANCE IN HUMAN SUBCUTANEOUS ADIPOSE TISSUE

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Objective: Severe insulin resistance is a defining attribute of gestational diabetes mellitus (GDM). In our previous studies, we have shown that adipose tissue from GDM women is associated with post-receptor defects in transduction of the insulin signalling pathway, specifically, decreased expression of insulin receptor substrate (IRS)-1 and glucose transporter (GLUT)-4 and increased phosphoinositide-3 kinase (PI3-K) p85α . Current dogma suggests that hormones secreted during pregnancy, are mediators of the insulin resistance associated with GDM. Thus, the purpose of this study was to determine the effect of human placental lactogen (hPL), a hormone only secreted during pregnancy, on the gene expression of the insulin signalling pathway in human subcutaneous adipose tissue.

Method: Human subcutaneous adipose tissue (n=3) was obtained from normal healthy women (BMI<25) undergoing Caesarean section. Tissues were dissected and incubated in serum-free DMEM (~100mg weight/well) in the absence/presence of 1μM hPL for 18hrs, and then stimulated with 100nM insulin for 45mins. Tissues were collected and the effect of hPL on insulin receptor (IR)-β, IRS-1, IRS-2, PI3-K

p85α and PI3-K p110 and GLUT-1 and -4 were determined by quantitative RT-PCR. Conditioned media was measured to determine glucose uptake by the tissue. Statistical analysis was performed using one-way ANOVA where $p<0.05$ was significant.

Results: RT-PCR demonstrated that hPL treatment significantly decreased insulin-induced IR-β, and GLUT-4 mRNA expression. In contrast, hPL treatment increased insulin-stimulated PI3-K p85α mRNA expression. Insulin-stimulated glucose uptake from adipose tissue treated with hPL was also significantly reduced.

Conclusion: This study demonstrates that hPL interferes with both the initial steps and downstream transduction of the insulin signalling pathway. hPL may play a role in the aetiology of GDM.

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PRO-INFLAMMATORY CYTOKINE RELEASE AND MATRIX METALLOPROTEINASE ACTIVITY IN RESPONSE TO PPAR GAMMA-ACTIVATION IN LPS-STIMULATED HUMAN GESTATIONAL TISSUES

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Objective: Pro-inflammatory cytokines and extracellular matrix (ECM) remodelling enzymes (matrix metalloproteinase (MMPs)) contribute to the onset of human labour. Cytokines and MMPs are associated with peroxisome proliferator-activated receptor gamma (PPARg) transcriptional control. PPARs are anti-inflammatory transcription factors that are proposed to promote uterine quiescence and thus suppress inflammatory-mediated labour processes. The hypothesis to be tested is that PPARg ligand activation will decrease pro-inflammatory cytokine release and MMP enzyme activity in human gestational tissues. Methods: Placenta, amnion and choriodecidua collected from women at term undergoing elective Cesarean section for singleton delivery were used in explant cultures (n=3 in duplicate). Tissues were incubated in the presence or absence of potent PPARg ligand GW1929 (12.5 and 25mM) in the presence of LPS. Tissues were incubated for 6 hr at 37°C, 8% O_2 and 5% CO_2. Incubation media was assayed for the release of IL-6, IL-8 and TNF-a (choriodecidua only) by ELISA. Incubation media and tissue lysates were assayed for MMP enzyme activity using gelatin zymography. Data was analysed by one-way ANOVA (LSD correction) and statistical difference was indicated by $p<0.05$. Results: GW1929 significantly attenuated LPS-stimulated IL-8 release from amnion (25mM) and choriodecidua (12.5 and 25mM) ($p<0.05$). There was however no effect of GW1929 treatment on LPS-induced IL-8 release from placenta. For all three tissues, although 25mM GW1929 reduced LPS-induced IL-6 release, this did not reach statistical significance. LPS-stimulated TNF-a release from choriodecidua was significantly reduced in the presence of 25mM GW1929 ($p<0.05$). GW1929 at both concentrations significantly inhibited LPS-stimulated active-MMP-2 enzyme activity in placental lysates ($p<0.05$). There was no effect of GW1929 treatment on LPS-stimulated MMP enzyme activity secreted from all three tissues. Conclusion: Treatment of term human gestational tissue explants with PPARg-specific agonist, GW1929, hinders pro-labour mediators (IL-8, TNF-a and MMP-2), therefore supporting the hypothesis that PPARs may help to maintain uterine quiescence via pro-labour mediator suppression.

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ROLE OF THE VESICULAR CHLORIDE TRANSPORTER CLC-3 IN SECRETION FROM ENDOCRINE CELLS

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The acidification of secretory vesicles is essential for proper loading and secretion of hormones such as insulin and adrenaline. In order to maintain loading, a negative charge is required to enter vesicles and this is commonly thought to occur via chloride transport. In this study, we illustrate the role of the chloride transporter/channel, CLC-3, in the loading of secretory vesicles and in regulating exocytosis. ClC-3 is an intracellular chloride transport protein known to reside on endosomes and synaptic vesicles. However, the endogenous protein has been notoriously difficult to detect in immunohistological experiments because of the lack of reliable antibodies. Using newly generated antibodies, we now examine its expression pattern at the cellular and subcellular level. In all tissues examined, immunostaining indicated that ClC-3 is a vesicular protein, with a prominent expression in endocrine cells like adrenal chromaffin cells and pancreatic islet cells. In line with a possible function of ClC-3 in regulating vesicle trafficking or exocytosis in those secretory cells, patch clamp capacitance measurements and carbon fibre amperometry indicated that exocytosis of large dense-core vesicles (LDCVs) was decreased in chromaffin cells from ClC-3 knock-out mice. However, immunohistochemistry complemented with subcellular fractionation showed that ClC-3 is not detectable on LDCVs of endocrine cells, but localizes to endosomes and synaptic-like microvesicles in both adrenal chromaffin and pancreatic beta cells [1]. This observation points to an indirect influence of ClC-3 on LDCV exocytosis in chromaffin and beta cells, as opposed to direct effect previously published [2]. This possibly occurs by affecting an unidentified intracellular trafficking step and illustrates a formerly unidentified level of cross-talk between vesicle species within these cells.

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LIGAND-INDUCED ACTIVATION OF PPARA AND PPARΓ UPREGULATES EXPRESSION OF ANTIOXIDANT DEFENCES IN PLACENTAL BEWO CELLS

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Placental oxidative stress is a major contributor to placental-related pathologies, including preeclampsia and intrauterine growth restriction, and is characterised by elevated production of reactive oxygen species (ROS). Protection against excessive ROS accumulation is provided by antioxidant enzymes, including superoxide dismutase isoforms (SOD1 and SOD2) and catalase, which catalyse the inactivation of ROS. Furthermore, uncoupling protein-2 (UCP2) limits the production of ROS by an as yet unidentified mechanism. The PPARγ and PPARα agonists, rosiglitazone and GW7647 respectively, have been proposed to exhibit antioxidant activity. In this study we hypothesised that these PPAR ligands alter expression of the antioxidant enzymes within placental BeWo cells, under normal culture conditions and following syncytialisation with 20 μM forskolin (FSK) for 72h. Normal and syncytialised BeWo cells were incubated with either rosiglitazone (0.3, 1, 3 or 10 μM), GW7647 (3, 10, 30 or 100 nM) or vehicle for 24 h and quantitated for mRNA expression of SOD1, SOD2, catalase and UCP2 by qPCR. Rosiglitazone upregulated expression of SOD1 (1.5-2-fold, P= 0.033), catalase (1.3-5-fold; P = 0.013) and UCP2 (1.2-2-fold; P < 0.001) in a dose dependent manner from 1 μM, while GW7647 upregulated catalase (2-fold, P = 0.027) at 100 nM only. Syncytialisation of BeWo cells by FSK upregulated expression of SOD1 (1.5-fold, P<0.01), SOD2 (1.6-fold, P = 0.03) and PPARγ (1.5-fold, P<0.001), consistent with higher antioxidant capacity in the syncytiotrophoblast layer of the placenta. Interestingly, syncytialisation attenuated the upregulation of SOD1 by rosiglitasone and catalase by GW7647, and resulted in a GW7647-dose dependent decrease in SOD1 expression (P = 0.018). These data suggest that activation of PPARγ or PPARα may increase the capacity of BeWo cells to inactivate ROS; a likely mechanism for the antioxidant properties attributed to the PPAR ligands. Furthermore, treatment of BeWo cells with PPARγ ligands mimics some of the phenotypic changes that occur during syncytialisation.

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LEVELS OF EXPRESSION OF COMPONENTS OF THE RENIN-ANGIOTENSIN SYSTEM (RAS) IN FETAL MEMBRANES, DECIDUA AND PLACENTA AND THE EFFECTS OF GENDER AND LABOUR

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Human intrauterine tissues and amniotic fluid contain prorenin. Prorenin generates angiotensin I (Ang I) from angiotensinogen (Agt) and stimulates cell signalling pathways directly when bound to the prorenin receptor ((P)RR) (1). To determine the expression of the RAS in human gestational tissues, term (37-41 weeks) amnion, chorion, decidua and placenta were collected from 47 women during elective caesarean section or after spontaneous labour. Real time RT-PCR for measuring prorenin, (P)RR, Agt, angiotensin converting enzyme-1 (ACE1), ACE2 and angiotensin type I receptor (AT1R) mRNAs was performed. Before labour, prorenin mRNA abundance in decidua from pregnancies carrying a female fetus was greater than in decidua from 'male' pregnancies (P=0.007). After labour, decidual prorenin mRNA levels were lower in 'female' pregnancies, so there was no difference between 'male' and 'female' pregnancies (P=0.12). Decidua had the highest levels of prorenin, Agt and ACE1 mRNAs (P<0.008). ACE2 mRNA in decidua and placenta were similar. (P)RR mRNA was expressed in all four tissues but was most abundant in placenta (P<0.001). In amnion, prorenin, Agt, ACE1 and ACE2 and AT1R mRNA levels were low (median values relative to decidua (100%) were 0.3%, 0.7%, 3.6%, 12% and 0.2% respectively). RAS mRNA levels in the chorion were higher than amnion (relative to decidua; 12.6%, 27%, 16%, 41% and 45%). Placental prorenin mRNA was low (15% relative to decidua) but Agt (37%), ACE1 (61%), ACE2 (93%) and especially AT1R (4432%) mRNAs were high. These results suggest that in amnion the RAS may exert its effects independently of Ang II, while in placenta RAS effects could be mediated through the AT1R. In decidua, Ang 1-7 may be important since AT1R levels are low. Tissues exposed to high extracellular levels of prorenin (placenta and amnion) have low levels of prorenin mRNA, suggesting paracrine sources of prorenin act at these sites.

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PLACENTAL MICRO RNA EXPRESSION IN PREGNANCIES COMPLICATED BY ASTHMA

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Background: Micro RNAs (miRs) are non-coding small RNAs and act as important post-transcriptional regulators of gene expression by altering the abundance or translational efficiency of mRNAs. miR- mediated gene regulation is important for normal physiological and cellular functions and they play key role in immune responses, differentiation and developmental processes. Recent evidence suggests important role of miRs in pathological conditions. We have previously identified sex specific differences in placental gene expression associated with strategies for optimal growth and fetal survival in the presence of maternal asthma. The current study sought to examine miR expression in male and female placentae of pregnancies complicated by asthma and in the event of an asthma exacerbation.

Aims/Hypothesis: We hypothesise that post-transcriptional regulation of genes by miRs may play an important role in conferring sexual dimorphism in placental gene expression during development.

Methods: RNA was extracted from male (n=12) and female (n=12) placentae from pregnancies complicated by asthma and miR analysis was conducted. miRGEN target prediction algorithm database (http://www.diana.pcbi.upenn.edu/cgibin/miRGen/v3/Targets.cgi) was used to identify predicted targets for identified miRs. Target genes were then imported into Ingenuity Pathways Analysis (IPA) software to identify functional networks of differentially expressed miRs.

Results: Seventy five miRs were differentially expressed between male and female placentae based on a *P*-value of less than 0.05. Maternal asthma exacerbation was associated with 24 differentially expressed miRs in female placenta compared with males. This included miR-203 and miR-223 which are associated with chronic inflammatory diseases and cytokine responses. Pathway analysis identified networks involved in glucocorticoid receptor signalling, cytokine signalling, apoptosis, cellular growth and differentiation.

Conclusions: There are differentially expressed miRs in male and female placentae in pregnancies complicated by chronic asthma and an acute exacerbation which target genes involved in cytokine expression and other immune pathways. These data indicate placentae act in a sex specific manner in response to an adverse event during pregnancy.

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DEVELOPMENT OF MYOMETRIAL CONTRACTILITY IN HUMANS IS ASSOCIATED WITH PHOSPHORYLATION OF COFILIN AND CALDESMON

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BACKGROUND: Cofilin is an actin binding protein which when dephosphorylated is responsible for depolymerising F-actin in smooth muscle. Upon phosphorylation cofilin is inactivated, thus promoting F-actin formation and the development of tension. Similarly, phosphorylation of caldesmon relieves its inhibitory action on myosin ATPase, while at the same time promoting cross-bridge cycling and thus once again promoting tension development in smooth muscle. Despite being fundamental mechanisms regulating tension development in smooth muscle, the role of these proteins is yet to be analysed in human myometrium.

HYPOTHESIS/AIMS: We hypothesised that cofilin and caldesmon regulate tension development in human myometrium during the onset of labor. We aimed to test this hypothesis through comparing the phosphorylation status of cofilin and caldesmon before and after the establishment of spontaneous contractions in vitro.

METHODS: Human myometrial tissue was collected from term, non-labouring women. Tissue was dissected into strips and suspended in organ baths under tension. Transducers were used to observe the onset of spontaneous contractions. To highlight phosphorylation changes associated with contractions, strips were snap frozen; (i) prior to the onset of any contractions, (ii) at maximum contraction, and (iii) at maximum relaxation between contractions. Proteins were extracted, separated using SDS-PAGE and western transferred. Phospho-specific antibodies were then used to detect target proteins.

RESULTS: We observed that the onset of spontaneous contractions in vitro was associated with a 3-fold increase in caldesmon phosphorylation as well as a 26-fold increase in cofilin phosphorylation.

DISCUSSION: While cofilin and caldesmon have previously been reported as regulators of smooth muscle contraction, caldesmon was yet to be examined human myometrium while cofilin was yet to be examined in mammalian myometrium at all. Our novel findings provide evidence that both of these proteins are important regulators of myometrial contractility in humans, with likely roles during the onset of labor in vivo.

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PREVALENCE OF VITAMIN D DEFICIENCY IN PREGNANCY IN 2 AUSTRALIAN POPULATIONS

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Introduction: Pregnant women of ethnic origin with minimal sun exposure are recognised to be at risk of vitamin D deficiency (1,2). Correlation data on demographic factors, sun factors and vitamin D supplementation in determining vitamin D status in pregnancy is lacking (3,4,5).

Aim: To determine the prevalence and predictive factors of vitamin D deficiency in pregnant women attending antenatal clinics.

Design: Cross-sectional study of pregnant women between 15-28 weeks gestation from Canberra(n=83) and Campbelltown (n=104).Assessments included a standardised questionnaire, Fitzpatrick score and vitamin D levels. Data was analysed separately.

Results: Using the conservative cut point of 50nmol/L, the prevalence of vitamin D deficiency was 38.6% and 25.7% in Canberra and Campbelltown respectively. Majority had mild vitamin D deficiency (26-50nmol/L). Women of ethnic background constituted 27.6% and 16.9% in Campbelltown and Canberra respectively.

In Campbelltown well recognised risk factors including ethnicity, Fitzpatrick score, sun exposure and season were determinants of the vitamin D level both in univariate and multivariate analyses.

In Canberra absence of medical condition, spring season and lower skin exposure were determinants of low vitamin D levels in univariate analysis. On multivariate analysis higher body mass index, lower skin exposure and absence of tropical holidays were associated with vitamin D insufficiency.

In both populations vitamin D supplementation with at least 500IU/day was not associated with significantly higher levels of vitamin D. Highest prevalence was seen in spring.

Conclusion : Prevalence of vitamin D deficiency was 26%-39% in pregnancy. With a higher ethnic population (Campbelltown) traditionally recognised factors were determinants of vitamin D levels. In a predominantly Caucasian population (Canberra), apart from sun factors, BMI was a significant determinant of vitamin D level. In both populations vitamin D 500IU/day was not adequate. Routine vitamin D supplementation with higher dose than 500IU/day, particularly in obese pregnant women in colder months should be considered.

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A NEW MECHANISM FOR CRH-INDUCED IMMUNOSUPPRESSION DURING PREGNANCY

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Background: During pregnancy the placenta expresses the retroviral envelope proteins Syncytin-1 and Syncytin-2 (1, 2) and secretes the microvesicles known as exosomes with postulated immunomodulatory functions (3). The human placenta also secretes Corticotropin Releasing Hormone (CRH), which stimulates production of placental cAMP, a known inducer of Fas Ligand (FasL). cAMP also stimulates expression of Syncytin-1 (1) and Syncytin-2 (4).

Hypothesis: CRH upregulates the production of the immunosuppressive factors FasL, Syncytin-1 and Syncytin-2 in the placenta and stimulates the sorting of FasL and Syncytin-1 into placental exosomes.

Methods: BeWo cells were incubated in the presence of CRH (10-200nmol/L) or vehicle for 24 hours. mRNA levels of Syncytin-1, Syncytin-2 and FasL were analysed by real-time PCR. Protein levels of Syncytin-1, Syncytin-2 and FasL were analysed by Western Blotting. Exosomes were prepared from the supernatants of CRH treated BeWo cells using differential centrifugation and sucrose gradients. Exosomal protein was analysed by SDS-PAGE and Western Blotting. Human whole blood was incubated with lipopolysaccharide (10ng-100μg) in the presence and absence of the Syncytin-1 Immunosuppressive Peptide (ISU) (7.5-60μmol/l). TNF α levels were assayed by ELISA.

Results: Following 24 hour CRH (10-200nmol/L) treatment of the BeWo human placental cell line, real-time PCR analysis showed a significant upregulation of Syncytin-1 (50nmol/L, $p<0.01$), Syncytin-2 (50nmol/L, $p<0.01$) and FasL (50nmol/L, $p<0.01$) mRNA in a dose dependent manner. CRH also increased exosomal protein production. Analysis of the Syncytin-1 protein in an exosomal fraction showed a 3.2 fold increase following CRH treatment (50nmol/L, $p<0.05$). Syncytin-1 ISU led to immunosuppression as shown by a 35% decrease in the production of TNF a by peripheral mononuclear cells following addition of 30μmol/l peptide.

Conclusions: CRH regulates the placental production of the immunosuppressive factors; Syncytin-1, Syncytin-2, FasL and exosomes during pregnancy. This is a novel mechanism for the endocrine regulation of the maternal immune system during pregnancy.

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THE INFLUENCE OF ANTENATAL GLUCOCORTICOIDS AND FETAL SEX ON PRETERM PLACENTAL OXIDANT AND ANTI-OXIDANT STATUS

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Background: Sexual dimorphism in the incidence of patho-physiological pregnancy, neonatal morbidity/mortality and the response to antenatal glucocorticoids is well recognised. Oxidative stress is a central element in both patho-physiologic conditions precipitating preterm birth and common preterm neonatal morbidities. With glucocorticoids known to increase oxidative stress, we hypothesised placental oxidant and anti-antioxidant status would differ with fetal sex and antenatal steroid exposure.

Method: Placentae were collected from 28 women who delivered <29 weeks gestation. Oxidative stress (nitrotyrosine and protein carbonyl concentration), anti-oxidant enzyme activity (glutathione peroxidase and superoxide dismutase) and reactive oxygen species (nitric oxide (NO) and carbon monoxide (CO) concentration) where determined by ELISA and spectrophotometry. Steroid exposure was defined as delivery <72 or >72 hours after maternal steroid administration. Data was analysed by ANOVA

Results: Sex-specific differences were evident in oxidative stress with levels greater in males (protein carbonyl p=0.04, nitrotyrosine p=0.018). Nitrotyrosine was influenced by steroid exposure (p=0.04), being lower in infants born <72 hours after steroid exposure. An interaction between sex and steroid exposure was seen for placental NO (p=0.02) with levels higher in males than females if born <72 hours after steroid exposure (p<0.01) and, for females, higher in those born >72 after steroid exposure (p=0.01). Sex alone influenced CO (p=0.03) with levels higher in males. While superoxide dismutase activity was not influenced by sex or antenatal steroid exposure, female fetal sex was associated with higher glutathione peroxidise activity (p=0.05).

Conclusions: This data supports sex-specific differences in placental reactive oxygen species and markers of oxidative stress with non-uniform differences in anti-oxidant or pro-oxidant effects following antenatal steroid exposure. Male sex is associated with a higher incidence of adverse pregnancy and neonatal outcome which could be derived from greater susceptibility to oxidative stress.

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THE USE OF LUGOL'S IODINE FOR THE CONTROL OF SEVERE THYROTOXICOSIS IN PREGNANCY

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Fulminating thyrotoxicosis can be difficult to control in pregnancy. Addition of oral Lugol's iodine to propylthiouracil can rapidly control hyperthyroidism, but has not been well studied in pregnancy. We present two women (three pregnancies) with severe thyrotoxicosis treated with a combination of Lugol's iodine and PTU during pregnancy.

Case One (22 years) presented with thyrotoxicosis during her first pregnancy at 22 weeks gestation. Despite the addition of PTU 100mg tds her FT4 rose from 58 to 93 (9-23 pm/L) over two weeks. Oral Lugol's iodine was then begun and continued with PTU 100mg tds until delivery at term. The infant became hyperthyroid shortly after birth, requiring treatment with PTU from 2-6 weeks , and subsequent thyroxine therapy from 7 weeks until 10 months of age. Her physical and intellectual development was supervised until 15 months and was normal. A second pregnancy 2 years later was also characterised by difficult maternal hyperthyroidism and required the addition of Lugol's iodine to PTU in the last month of pregnancy. She delivered a healthy boy born at term who had central hypothyroidism. At last follow up at 3 months of age he was healthy and developing normally but still requiring thyroxine.

Case Two (21 years) presented with Graves disease in her first pregnancy at 9 weeks gestation. She was systemically unwell, requiring hospital admission and her FT4 was >160 (9-23 pmol/L). Lugol's iodine and PTU 100mg tds were started simultaneously. Six weeks later the iodine was ceased and she delivered a healthy infant at term. Her second pregnancy 2 years later was managed with PTU alone.

These cases illustrate the use of Lugol's iodine in pregnancy for the control of severe thyrotoxicosis. No adverse effects of therapy for the mother or infant were noted in this small case series.

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BMPR-IB NEUTRALIZATION IN MALE MICE REVEALS A REGULATORY ROLE FOR THE TYPE I RECEPTOR IN GONADOTROPHIN STIMULATED ANDROGEN SECRETION

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The reproductive system of males is regulated by endocrine gonadotrophin signaling which up-regulates testosterone production by the testis necessary for male germ cell development and maturation. Furthermore, increased androgen production facilitates the maturation of seminal vesicles which are extremely sensitive to androgens and have been used as an indirect but specific measure of testosterone production. A small but growing body of evidence also suggests major developmental roles for bone morphogenetic proteins (BMPs) and BMP receptors in the modulation of testosterone synthesis, germ cell maturation, integrity of reproductive tissues and epithelial secretory function all vital for normal reproductive capacity. In this study we investigated the effects of passive vaccination against BMPR-IB on the male reproductive system of immature mice.

Immature male mice (21 days old) were passively immunized against BMPR-IB via subcutaneous injections of 100 µl PBS containing anti BMPR-IB Ig in the absence and presence of equine chorionic gonadotrophin (eCG). The preparations where administered every day for six days. On the seventh day mice were killed by CO_2 asphyxiation and the testis and seminal vesicles were removed and weighed.

BMPR-IB immunization significantly reduced gonadotrophin stimulated weight gain of the testis in immature male mice. Additionally in gonadotrophin treated mice, BMPR-IB neutralization also caused a significant decrease in seminal vesicle weights.

Our study demonstrates that BMPR-IB enhances gonadotrophin stimulated maturation and development of the testis in immature mice. Gonadotrophin induced androgen production are in part mediated by BMPR-IB as determined by the inhibitory effect of BMPR-IB neutralization on seminal vesicle weights during the initiation phase of male reproductive development in mice.

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IDENTIFICATION OF G-PROTEIN COUPLED RECEPTORS (GPCRS), GPR40 AND GPR120 IN HUMAN ENDOMETRIAL CANCER CELL LINES AND THEIR POTENTIAL REGULATION OF CELL PROLIFERATION

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Epidemiological studies have previously revealed an association between dietary fatty acids and the incidence of endometrial cancer. In addition, evidences indicates that obesity, which is characterised by elevated circulating free fatty acids (FFAs) and hyperlipideimia is associated with increased reproductive cancer risks. GPR40 and GPR120, two G-protein coupled receptors, have recently been identified as functional receptors for medium- to long- chain FFAs. Long chain FFAs have previously been shown to play an important role in the regulation of cell proliferation and differentiation in human breast cancer cell line, MCF-7. In this study, we demonstrated that the human endometrial cancer cell lines, LDN, Ishikawa, HEC-1A and -1B differentially expressed both GPR40 and GPR120. Treatment of cells with oleic acid (C18:1) stimulated cell proliferation in a dose dependent manner. Similar increase in cell proliferation was observed when cells were treated with GPR40 and GPR120 agonist, GW9508, suggesting that the cell proliferation is mediated by GPR40 and/or GPR120 signalling pathway. To examine whether GPR activates any signal transduction in human endometrial cancer cells, we measured changes in intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$), which was elevated by oleic acid (10 µM) pre-mixed with 0.5% BSA. Our result showed that oleic acid caused a rapid and significant increase in $[Ca^{2+}]i$ and cell proliferation in endometrial cancer cell lines, suggesting that GPCRs are not only receptors that control hormone secretion, but also play an important role in the regulation of cancer cell proliferation. In conclusion, this is the first study to identify the mRNA expression of GPR40 and GPR120 in human endometrial cancer cell lines and FFA induced cell proliferation is likely to be mediated by these GPCRs.

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THE MINERALOCORTICOID RECEPTOR: EXPLORING LIGAND-SPECIFICITY USING PEPTIDE PHAGE DISPLAY

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The mineralocorticoid receptor (MR) is a member of the nuclear receptor superfamily and is essential for controlling sodium transport in epithelial tissues such as the kidney and colon. Moreover, it is also present in other non-epithelial tissues including the cardiovascular system where it plays a role in the pathophysiology of cardiac failure. The MR is unique in that it can be activated by both mineralocorticoids (aldosterone, deoxycorticosterone) and glucocorticoids (cortisol). A challenge in understanding transcriptional regulation by the MR is to determine how ligand and tissue specificity is achieved. Over the past decade, it has become clear that coregulator proteins are critical for nuclear receptor-mediated gene expression. A subset of these coregulators may confer specificity to MR-mediated responses as has been shown for other nuclear receptors such as the estrogen and androgen receptors.

We hypothesize that different physiological ligands can induce distinct MR conformations which permit distinct coregulator interactions. These subtle changes may underlie differential gene regulation by these ligands. We used phage display to screen a library of 10^8 small (19 aa) peptides for their interaction with the MR in the presence of aldosterone, cortisol or deoxycorticosterone. The isolated peptides (n = 173) were assessed for functional interactions with the MR by mammalian two-hybrid and transactivation assays.

We have identified a unique motif, MPxLxxLL, in peptides that strongly bind MR in the presence of aldosterone. This reflects an area of the MR-coregulator interface that may be amenable to ligand-specific blockade by peptidomimetics and will be further explored using transactivation interference assays. We were unable to identify ligand-specific peptides for cortisol or deoxycorticosterone, using this approach. The identification of this consensus motif provides proof of concept that phage display is useful in studying the molecular signaling of MR. The identification of more ligand-specific peptides may permit the rational development of peptidomimetics which act as specific MR antagonists for the treatment of cardiac failure.

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ASSESSMENT OF THE REPRODUCTIVE TOXICITY OF COMPOUNDS FROM THE MARINE MOLLUSC *DICATHAIS ORBITA*

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Dicathais orbita, a marine gastropod, is a source of Tyrian purple which has been used in folk medicine and homeopathy to treat a range of gynaecological disorders, including uterine cancer. The chemical pathway to Tyrian purple involves the brominated indole compounds tyrindoleninone and tyrindolinone which prevent proliferation in human lymphoma and breast cancer cells. We aimed to examine the effects of extracts from *D.orbita* on human granulosa cells and compare with reproductive cancer cell lines.

Compounds were solvent extracted from the hypobranchial glands of specimens of *D.orbita* and semi-purified on a silica column. The compounds in selected fractions were identified by LC/MS. Primary-derived human granulosa cells were isolated from follicular aspirates of women undergoing ART and compared with the human reproductive cancer cell lines KGN and OVCAR-3. Granulosa cells

(10,000cells/well), KGN and OVCAR-3 cells (20,000cells/well) were exposed to fractions (0.005-1mg/ml) for 4, 24 and 48h. Cell viability was determined by the MTT assay (0.5mg/ml) and hormone synthesis by radioimmunoassay.

Three fractions were selected: fraction 1 contained a mixture of brominated indoles, fraction 2 tyrindoleninone and tyrindolinone and fraction 3, contained 6-bromoisatin. After 48h, fraction 2 (0.05mg/ml) decreased cell viability by 88 and 51% in KGN and OVCAR-3 cells respectively, but by only 9% in primary granulosa cells, and had no effect on progesterone or oestrogen synthesis. Fraction 3 (0.05mg/ml) reduced KGN and OVCAR-3 cell viability by 49 and 51% after 48h whereas, granulosa cell viability was only reduced by 13%. However, progesterone synthesis by granulosa cells was also inhibited by fraction 3.

The 3 *Dicathias* fractions all showed greater activity towards the KGN and OVCAR-3 cell lines in comparison to the primary granulosa cells. Fraction 2 which contained tyrindoleninone was the most promising candidate with the greatest anti-proliferation activity towards the reproductive cancer cell lines without significantly affecting primary cells.

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ESTROGEN DEPENDENT GENE EXPRESSION IN THE MOUSE OVARY

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Estrogen (E_2), a gonadal hormone, plays a pivotal role in regulating the female reproductive system. However, the number and type of all ovarian genes influenced by estrogen remain to be elucidated. In this study, we have utilized wild-type (WT) and aromatase knockout (ArKO; estrogen free) mouse ovaries as an *in vivo* model to profile estrogen dependent genes.

Both ovaries from each of 3 WT and 3 ArKO mice (C57B6/J129; 16 weeks old) were individually snap frozen in Ultraspec solution for RNA isolation . Three ug of total RNA from each individual ovary was analyzed by a microarray-based screen using Illumina Sentrix Mouse WG-6 BeadChip (45,281 transcripts).

Comparative analysis (GeneSpring) showed differential expression profiles of 456 genes influenced by E_2, with 295 genes up-regulated and 161 down-regulated by 2-fold or greater in the ArKO ovary. Genes previously reported to be E_2 regulated in ArKO ovaries (1) were identified, in addition to genes not previously reported to be expressed or E_2 regulated in the ovary. Of genes involved in 5 diverse functional processes (hormonal, reproduction, sex differentiation and determination, apoptosis and cellular) 67 had estrogen-responsive elements (ERE): Up-regulated; *Ada, Adam23, Agt, Ank2, Ace, Angptl6, Bcl2, Cga, Cldn10, Col12a1, Col8a1, Cyp1b1, Cyp27a1, Cidea, Cpz, Ddr1, Dmkn, Fbln7, Gdnf, Gpr125, Hsd11b1, Lama2, Lamb2, Lamc3, Lpar3, Ltbp2, Myo7a, Nuak2, Ncam1, Pcsk6, Pdpn, Pdgfra, Ppl, Sort1, Sox9, Stard13, Serpine2, Timp2, Tnfrsf21, Tns4, Ttyh1, Wdr92, Wdh;* Down-regulated; *Aoc3, Cyp17a1, Cyp51, Cables1, Ednrb, Foxo1, Hsd17b7, Heph, Igsf11, Irs1, Ihh, Ltbp1, Megf10, Nrcam, Oca2, Onecut2, Rasgrp1, Sdc1, Serpina3g, Scarb1, Sesn3, Sfrp4, Timp1, Vldlr*.

This defines the transcriptome regulated by E_2 in the mouse ovary, allowing further analysis to increase the fundamental knowledge pertaining to how E_2 influences follicular development and other ovarian functions.

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(1) Britt et al., Biol Reprod. 71(5):1712-23 (2004)

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POLYCYSTIC OVARY SYNDROME: A BIOPSYCHOSOCIAL UNDERSTANDING IN YOUNG ADULT WOMEN TO IMPROVE KNOWLEDGE AND TREATMENT OPTIONS

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Objective: To assess the biopsychosocial features of polycystic ovary syndrome (PCOS) in young women
Design: Observational cross-sectional study

Setting: Community based clinical research

Population: Young women aged 18-25 with (n=22) or without (n=24) PCOS (22.41 ± 0.39 vs 21.95 ± 0.47 years, p=0.46; 29.17 ±1.54 vs 22.05 ± 0.83 kg/m2 , p=0.0003).

Methods: Internet or mailed questionnaire survey

Main outcome measures: Physical reproductive and metabolic features of PCOS, quality of life, anxiety, depression and perception of risk and fears of future health complications.

Results: Women with PCOS presented with worsened quality of life (p=0.033) and greater anxiety (p=0.01) and depression (p=0.023). On adjustment for BMI these relationships were no longer significant with a trend for worsened quality of life (p=0.077), depression (p=0.057 and anxiety (p=0.102). Women with PCOS perceived themselves to be at greater risk for obesity (p=0.012) and infertility (p<0.001), perceived greater importance in reducing future risk of prediabetes (p=0.027), gestational diabetes (p=0.039), type 2 diabetes (p=0.01), heart disease (p=0.005), PCOS (p=0.015), obesity (p=0.0007) and infertility (p=0.024) and had greater fears about future health related to weight gain (p=0.045), loss of femininity (p=0.035), loss of sexuality (p=0.003) and infertility (p=0.019) and trends towards greater fear

with regards to no more children (p=0.09) and type 2 diabetes (p=0.08) compared to women without PCOS. Conclusion: Worsened quality of life, anxiety and depression in young women with PCOS is partially mediated by elevated BMI. Future risks of metabolic conditions are less likely to be perceived as immediate concerns compared to weight gain and infertility.

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GONADOTROPHIN INHIBITORY HORMONE (GNIH) NEURONES ARE NOT ACTIVATED BY PSYCHOSOCIAL STRESS IN THE EWE

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Reproduction is inhibited by stress, however, the mechanisms and mediators by which this occurs remain unknown. It has been suggested that there may be a factor produced in the brain that contributes to reduced gonadotrophin secretion, as occurs during stress. One potential mediator is the neuropeptide gonadotrophin inhibitory hormone (GnIH) which has been shown to rapidly suppress gonado trophin secretion (*in vivo* and *in vitro*) and is, therefore, proposed to be the first negative regulator of the reproductive system (1). Since GnIH is produced in the brain and suppresses gonadotrophin secretion, GnIH may mediate the inhibitory effects of stress on reproduction. If GnIH has this role, then it would be expected that GnIH neurones would be activated during stress. We tested this hypothesis in ovariectomised ewes using the psychosocial stressor, isolation/ restraint. Ewes were randomly allocated to control or stress groups (n=5/ group). Isolation/ restraint stress was imposed for 90mins following control sampling for 4h while control ewes were sampled continuously for 5.5h. At the conclusion of the experiment, all ewes were killed and the brains perfused and collected. Immunohistochemistry was conducted for GnIH cells in the paraventricular nucleus (PVN) and dorsal medial hypothalamus (DMH) regions of the hypothalamus. As expected, plasma concentrations of cortisol were increased significantly ($P<0.05$) by stress. GnIH neurones were found in the PVN/ DMH regions of all ewes. On average, 51% of these were double labeled for Fos. The mean (±SEM) percentage of GnIH cells double labeled for Fos did not differ significantly between control (42.2±3.6%) and stress (48.7±16.0%) ewes. In conclusion, GnIH neurones are not activated by 90mins of psychosocial stress in ovariectomised ewes suggesting that this neuropeptide may not mediate the inhibitory effects of this stressor in sheep.

(1) Tsutsui et al, 2000. Biochemical and Biophysical Research Communications, 275, 661-67.

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APOPTOSIS IN PREGNANT MYOMETRIUM AND ITS ROLE IN LABOUR

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Context: Following delivery the uterus which has grown dramatically during pregnancy undergoes an equally dramatic involution. It has been assumed that this process begins following delivery of the infant. During recent histopathological studies of human myometrium obtained at term prior to the onset of labour we noticed that many myometrial cells demonstrated pyknotic nuclei suggestive of apoptosis. Objective:In this study, we investigated cell signalling pathways causing cell death in term human myometrium. Methods:Myometrial tissues were collected from elective or emergency caesarean sections. Tissues were immediately frozen in liquid nitrogen and stored at -80° C. These tissues were used for RNA and protein extraction. We performed realtime quantitative PCR and western blots for caspase-3 in both labouring and non-labouring myometrium. We performed immuno-histochemistry for activated Caspase-3 in paraffin embedded myometrial sections. We also performed western-blot for GAPDH, which is over-expressed in apoptosis. Results: Real-time PCR data showed that mRNA for Caspase-3 was expressed in higher amounts in labouring than non-labouring myometrium. This data does not correlate with our westernblot data which showed that protein for caspase-3 (~35 kD) was expressed in similar amounts in labouring than non-labouring myometrium. Western-blot showed the presence of activated cleaved caspase-3 (~17 kD) in some labouring samples but not in non-labouring samples. Additionally, we found that protein expression for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is higher in labouring myometrium than in non-labouring samples. GAPDH is an important regulator of the caspase independent cell death cascade. Cytotoxic stimuli, via nitric oxide generation, lead to the binding of GAPDH to the protein Siah1, translocation of GAPDH-Siah1 to the nucleus, and ultimately cell death. Conclusion:Both caspase dependent and caspase independent pathways are operating in myometrial cells undergoing apoptosis at term. The apoptosis may indicate a process of myometrial remodelling occurring during labour that may impact on myometrial function during labour.

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KIT LIGAND EXPRESSION AND REGULATION IN HUMAN OVARIAN GRANULOSA CELLS

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Female androgen excess is strongly associated with polycystic ovaries (PCO), but the contribution of androgen receptor (AR) signalling to development of this ovarian pathology is unknown. We have previously shown that levels of kit ligand (KITL) protein are dramatically increased in human PCO (ESA 2008), perhaps due to excessive stimulation of AR action *in vivo*. In this study we tested the hypothesis that KITL is an AR-regulated gene in human granulosa cells and characterised KITL protein isoform expression in human ovarian tissues at different stages of folliculogenesis. Cumulus granulosa cells were obtained from women receiving infertility treatment and treated ± 5α-dihydrotestosterone (DHT; 1-10 nM) for 6 hours in 6 independent experiments. The human granulosa tumour cell line, KGN, was treated ± DHT (1-100 nM) for 6 and 24 hours. Steady-state mRNA levels of KITL-1 and KITL-2 were determined by RT-qPCR. Western blotting was performed on protein lysates of KGN cells, human cumulus and mural granulosa cells from pre-ovulatory follicles, and human ovarian cortex containing primordial and primary follicles. Treatment with DHT did not alter KITL-1 or KITL-2 mRNA levels in cumulus granulosa or KGN cells under any conditions, and did not alter KITL protein levels in KGN cells. Ovarian cortex and KGN cells predominantly expressed KITL-2 protein while cumulus and mural granulosa cells primarily expressed KITL-1. In conclusion, although KITL has been identified as a candidate AR-regulated gene in mouse granulosa cells, we found no evidence for this in human granulosa cells. It is possible that a factor absent in our culture system but present *in vivo* is necessary to facilitate androgenic effects on KITL expression. The mechanism that causes increased KITL protein expression in PCO remains unknown, but we have shown that this is likely to involve KITL-2 at preantral stages and KITL-1 at pre-ovulatory stages of folliculogenesis.

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EFFECTS OF THE CYANOTOXIN CYN, ON HUMAN GRANULOSA CELL VIABILITY, HORMONE PRODUCTION AND PROTEIN SYNTHESIS

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Cylindrospermopsin (CYN) produced by the blue-green algae *Cylindrospermopsis raciborskii* is a hepatotoxin. CYN confers its toxicity via two mechanisms; cytochrome P450- metabolism (CYP450) and by inhibition of protein synthesis (PS). In mouse hepatocytes 0.5µM CYN inhibited PS by 4h in the presence of CYP450 inhibitors. GC may be sensitive to toxic effects of CYN as they have CYP450 enzymes for production of progesterone and estradiol and also undergo protein synthesis. GC can be stimulated by human chorionic gonadotropin (hCG) to increase P_4 production by upregulating steroidogenic enzymes. GC were isolated from women undergoing *in vitro* fertilization and cultured in DMEM/F12 + 10 % FCS at 37^{o}C with 5% CO_2 in 24- well plates. After an initial 24h adherence period, GC (40×10^3 cells/well) were incubated with 1µCi [^{3}H]-Leucine/mL medium ± 5µM CYN ± 1000mIU/mL hCG for 2, 4, 6 or 24h, after each time point the media was collected and stored for radioimmunoassay determination of steroid hormone concentrations. GC were then precipitated with ice-cold 10% TCA. Pellets were digested in 0.3M NaOH. Leucine incorporation reflected protein synthesis and was quantified by scintillation counting and results were expressed as cpm/µg DNA. Protein synthesis was significantly inhibited after 6h exposure to 5µM, $p<0.05$. Furthermore, there was a dose dependent decrease in progesterone production and protein synthesis after exposure to increasing CYN concentrations (0.1, 0.3, 1.0 or 3.0µM) for 6h. There was significant inhibition in PS with 3µM CYN after 6h but not with hCG-stimulated GC. These results suggest that PS is delayed in reproductive cells in comparison to mouse hepatocytes and a much higher CYN concentration is required to inhibit PS. The ability of CYN to decrease steroid hormone production and protein synthesis may indicate that it has the potential to disrupt normal reproductive cell functionality and development.

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A GLOBAL ANALYSIS OF ANDROGEN-REGULATED PROTEINS IN RAT TESTIS

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The effect of androgens on the testicular proteome is unknown, however androgens are essential for the completion of meiotic division and for survival of pachytene spermatocytes. This study aimed to identify androgen-regulated proteins in rat pachytene spermatocytes using a proteomic approach.

Pachytene spermatocytes were isolated by elutriation from controls rats, androgen-suppressed rats (8wks), androgen-suppressed and antagonist-treated rats, and androgen-replaced rats (4d) (all n=4/group). 2D-PAGE analysis with DIGE minimal dye labelling was conducted on 24cm pI 4-7gels, from which differentially expressed proteins were assessed by SameSpots software and identified by mass spectrometry using MALDI-Tof and LC-MS. Selected spots were validated by 2D-western blot analysis and immunohistochemistry.

263 significantly different ($p<0.05$) spots were recognised. Principle components analysis identified 4 distinct spot groups, indicating unique androgen-regulated protein expression profiles. Bioinformatics revealed proteins significantly associated with several Gene Ontology pathways relevant to pachytene spermatocytes. These included germ cell development, spindle assembly, cell cycle and apoptosis, protein transport, and chaperone and scaffolding functions. In particular, proteins involved in apoptosis were identified, such as

Smac/Diablo and PDIA3, and three 14-3-3 isoforms not previously described in spermatocytes. In addition, proteins with a potential role in regulation of meiotic division were androgen-regulated, including Ppp2r5e, the 14-3-3 proteins, tubulins, importinβ3, and centractin.

2D-western blot analysis also revealed that androgens can regulate post-translational modification of particular proteins, for example ATP-dependent RNA helicase DDX4 was resolved into 13 separate spots of which three were confirmed to be differentially regulated ($p<0.05$).

It is concluded that androgens regulate proteins at multiple levels in pachytene spermatocytes, including changes in protein expression and post-translational modification, with the latter presumably reflecting site-specific functions. The androgen-regulated proteins identified have putative roles in cell survival and meiotic division, and provide clues as to the mechanisms by which androgens regulate spermatogenesis.

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CYSTEINE-RICH SECRETORY PROTEIN (CRISP)-4 INHIBITION OF THE TRANSIENT RECEPTOR POTENTIAL MELASTATIN MEMBER 8 (TRPM8) ION CHANNEL: INVOLVEMENT IN SPERM FUNCTION AND IMPLICATIONS FOR MALE FERTILITY

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The Cysteine-Rich Secretory Proteins (CRISPs) are expressed abundantly in the male reproductive tract and to a lesser extent in other tissues. CRISPs are two domain proteins, with a conserved but evolutionary diverse N-terminal domain and a C-terminal domain, which we have shown inhibits the function of ion channels. CRISPs in the reproductive tract regulate ion channels and are involved in adhesion between the sperm and the oocyte. Their over-expression in other tissues is related to immune function and with the onset of prostate disease. As such, the detail of CRISPs biological functions and their roles in the maintenance of health and the onset of disease is of great importance. Within this study, we have focused on the characterization of CRISP4, which is secreted from the principal cells in the epididymis and surrounds sperm. We hypothesized that CRISP4 regulates specific ion channels in the plasma membrane of sperm and controls aspects of sperm maturation. To investigate this hypothesis, we have used electrophysiology of sperm to identify specific ion channels which are regulated by the C-terminal domain of CRISP4. Through patch clamp techniques, electrophysiology and the application of ion channel agonists and antagonists, we have demonstrated that CRISP4 inhibited the transient receptor potential (Trp) melastatin member 8 (M8) ion channel. We have confirmed inhibition of TRPM8 in transfected CHO cells and have demonstrated a functionally important role of TRPM8 in sperm function assays in the presence of TRPM8 agonists (icilin) and CRISP4. Our results show that sustained activation of TRPM8 significantly reduced the progesterone-induced acrosome reaction. This affect was reversed by the application of CRISP4. TRPM8 is the cold receptor in the body and is up-regulated in numerous cancers, including in the prostate. Demonstration that CRISPs are an endogenous inhibitor of TPRM8 has implication in the regulation male fertility and of disease.

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TESTOSTERONE'S SHORT-TERM POSITIVE EFFECT ON LUTEINISING HORMONE SECRETORY BURST MASS AND ITS NEGATIVE EFFECT ON SECRETORY BURST FREQUENCY ARE ATTENUATED IN MIDDLE-AGED MEN

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Background: Testosterone (T) production declines and LH pulses become smaller and more frequent from middle-aged men. The mechanisms underlying these changes are not known. Rationale. Small frequent LH pulses in middle-aged men could reflect feedback by systemic T. Hypothesis. Middle-aged disrupts negative feedback by T on selected facets of LH secretion. Subjects and Setting. Healthy men studied at an academic medical centre. Methods. Blockade of gonadal steroidogenesis and graded transdermal addback of T doses of 0, 2.5, 5 or 7.5 mg/day, designed to span the castrate to physiological range of T concentrations in each of 23 healthy men ages 19-71 yr. Quantification of 12-h basal and pulsatile LH secretion (92 time series) using a mathematically and empirically justified deconvolution method. Results. Stepwise T supplementation from the hypogonadal through the eugonadal range repressed mean (12-h) LH concentrations ($P=0.001$). By regression analysis, age attenuated the capabilities of increasing T concentrations to increase LH secretory burst mass ($P<0.0001$) and decrease LH secretory burst frequency ($P=0.025$). Age did not alter T's feedback on basal LH secretion, interpulse regularity, the waveform of LH secretory bursts or the slow half life of LH. Conclusion. Middle age impairs both the positive and negative actions of systemic T on pulsatile LH secretion in healthy men, thus potentially explaining earlier inconsistencies in feedback studies based upon single-sample mean LH concentrations. Longitudinal studies will be required to elucidate the precise age dependence of inferred dual feedback failure.

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GLUCOCORTICOID RECEPTOR EXPRESSION AND EFFECT OF CORTICOSTERONE ON MOUSE PROSTATE

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Androgen (AR) and glucocorticoid receptors (GR) are co-expressed in the prostate stromal and epithelial cells where they are assumed to have opposing effects with androgens stimulating cellular proliferation and glucocorticoids acting as potent anti-proliferative agents. Potent synthetic glucocorticoids are used as a last resort palliative treatment of patients with incurable hormone-refractory prostate cancer. Yet glucocorticoid effects on prostate have not been well defined. The present study evaluated the expression of GR in the mouse prostate and effects of supraphysiological dose of corticosterone, the primary circulating glucocorticoid in rodents, on prostate epithelial proliferation and apoptosis as well as morphology in a mouse. Adult male mice at age of 8 weeks received weekly s.c. implants of slow-release pellets containing either 1.5 mg of corticosterone or placebo for 4 weeks. Corticosterone treatment had no significant effect on testis or ventral prostate weight while the dorsolateral prostate, anterior prostate and seminal vesicle weights were significantly increased by 24%, 45% and 35%, respectively, compared with placebo treated mice. Epithelial proliferation (% of PCNA positive cells) was significantly increased in anterior prostate of corticosterone treated males, while apoptosis (TUNEL) was not affected. Histological analysis demonstrated that corticosterone treatment significantly influenced prostate morphology with epithelium appearing highly disorganized with frequent formation of bridge-like structures and increase of basal cell population. Immunohistochemical analysis revealed that GR in the mouse prostate is mainly expressed in stromal and basal epithelial cells, and occasionally in the luminal epithelial layer. The GR expression was not affected by corticosterone treatment. These data demonstrate that the murine prostate, and particularly stromal and basal epithelial cells, are significantly influenced by glucocortcoid treatment and emphasize that further studies are warranted to better understand the glucocorticoid action in the prostate.

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POSTNATAL DEVELOPMENT AND DYNAMIC OF THE SECRETED LEYDIG CELL PEPTIDE HORMONE INSULIN-LIKE PEPTIDE 3 (INSL3) IN RODENTS

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Insulin-like peptide 3 (INSL3) is expressed and secreted in large amounts by the interstitial Leydig cells of the testis. Our previous studies in the human have shown that this expression is acutely independent of the HPG axis, and reflects simply the differentiation status and number of Leydig cells present. Insl3 therefore offers us an important new tool to assess Leydig cell functionality *in vivo* without interfering effects due to the influence of steroids or LH. We have developed a new highly sensitive and robust time-resolved fluorescence immunoassay (TRFIA) which is capable of measuring INSL3 down to a concentration of 20pg/ml in both rats and mice without any extraction or concentration. Using this assay, we have been able to accurately map the differentiation of adult Leydig cells through puberty in Sprague Dawley rats and show that unlike androgens there is a marked "overshoot" effect during the first spermatogenic wave, which only settles down to a steady adult value at 3 months. Furthermore, using fluid sampling techniques combined with efferent duct ligation, we show that not only do high levels of Insl3 accumulate in the interstitial fluid from where they pass to testicular venous blood and hence to the general circulation, but significant concentrations of Insl3 are able to cross the blood-testis barrier established by the Sertoli cells and enter the tubular compartment, efferent ducts and epididymal lumen. This is an important finding since we have shown earlier that the principal receptors for Insl3, known as RXFP2, are mainly located on post-meiotic germ cell stages, where they are believed to act as germ cell anti-apoptotic factors, and on epididymal cells.

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EFFECT OF CRYOPRESERVATION ON OVINE LUTEAL CELLS STEROIDOGENESIS

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Cryopreservation of ovine luteal cells is required to counter the restricted availability of primary derived luteal cells due to seasonal anestrus of Ovis aries. Cell line cryopreservation commonly utilises 10% dimethyl sulfoxide (DMSO) supplemented with 40% foetal calf serum (FCS) in medium. Embryo cryopreservation utilises 1,2-propanediol supplemented with 40% FCS.

Ovine corpora lutea are comprised of large (LLC) and small (SLC) luteal cell and epithelial cells. Luteinising hormone (LH) stimulates SLC progesterone production four fold, while dibutyryl-cAMP (db-cAMP), an intermediate in the LH pathway also stimulates progesterone production.

This study aimed to examine the effect of cryopreservation of ovine luteal cell on cell viability and progesterone synthesis in response to LH and db-cAMP.

For each preparation (n=4), 10 corpora lutea (CL) were isolated from ovaries obtained from cyclic ewes at an abattoir. CL were diced and disaggregated in collagenase (400U/ml), with red blood cells removed by Lymphoprep™.

Each preparation was split into 4 groups (1 fresh, 3 frozen); freezing preparations were 5 or 10% DMSO, or 10% 1,2-propanediol, supplemented with 40% FCS in DMEM medium and frozen (3 months).

Each preparation was exposed to db-cAMP (0 - 1000µM) or LH (0 – 200ng/ml) and progesterone synthesis and cell viability analysed using radioactive-immunoassay and MTT assay respectively after 24 hours.

In fresh cells progesterone production increased to a maximum 1.85 fold of fresh basal rate (350ng/ml) when treated with 100µM db-cAMP, while frozen cells maximum occurred in cells exposed to 1000µM (1.55 fold) for 5% DMSO and 0.85 fold for 10% DMSO exposed to 100µM db-cAMP. LH produced similar but reduced dose response in fresh and DMSO cells as db-cAMP, while 1,2-propanediol cells had maximum 3% of fresh basal rate.

In conclusion, 5 or 10% DMSO can be used to freeze ovine luteal cells, while 1,2-propanediol cannot due to progesterone production inhibition.

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VARIATION IN ADULT MURINE LEYDIG CELL TESTOSTERONE PRODUCTION BY DIFFERENT ISOFORMS OF EQUINE CHORIONIC GONADOTROPIN USING INTERSTITIAL CELL CULTURE

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Equine chorionic gonadotrophin (eCG) is a heterodimeric glycoprotein hormone secreted by the placental endometrial cups during the first third of gestation in the horse. Equine CG is heavily glycosylated with ~45% carbohydrate by weight. Previous research has shown that eCG is a highly heterogeneous molecule with significant differences in bioactivity between isoforms. The aim of this study was to investigate the in vitro testosterone response of adult murine Leydig cells to a large range of eCG isoforms.

The eCG isoforms were isolated from eCG preparations using iso-electric focusing (IEF) (liquid phase) which fractionated preparations into 10 pH ranges between pH 3 and pH 10. Immunoactivity of IEF fractions and starting material was measured with RIA. For the interstitial cell preparation, adult male mice were asphyxiated, testes removed, decapsulated, dispersed, strained and washed with DMEM:F12 0.1% BSA. The cell stock was then counted and diluted ready for culture at 10,000 cells/well. Cell viability was determined using trypan blue. Treatment eCG was diluted in culture media to give a final well concentration of 1.0 IU/ml. The cells were incubated at 32 degrees Celsius for 3 hours in 5.0% CO_2 in humidified conditions. Media was collected after the 3 hours and immediately assayed for testosterone using RIA.

All cells stimulated with 1.0 IU/ml eCG produced significantly more testosterone then control. The acidic and intermediate isoforms of eCG showed similar steroidogenic bioactivity compared with the un-fractionated starting materials. However, with the more basic eCG isoforms, testosterone production from interstitial cells was significantly greater.

These results indicate that the isoform composition of eCG has marked effects on steroidogenesis. Most notably, the basic isoforms of eCG were highly potent stimulators of testosterone production. Our study is consistent with previous studies which have shown greater in vitro bioactivity of the more basic gonadotrophin isoforms.

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STEROID PRODUCTION CONTROLLED BY PROTEIN ASSEMBLY ON MEMBRANES

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The steroid synthetic pathway includes a cascade of reactions which generate all classes of steroids. Some multi-step reactions have particular functional significance. This is well illustrated in the regulation of androgen production; the conversion of pregnenolone to dehydroepiandrosterone (DHEA) catalyzed by 17 α-hydroxylase/17,20-lyase (P450c17), a critical sex steroid. P450c17 is bi-functional, catalyzing both hydroxylation and lyase reactions, the latter enhanced by the presence of cytochrome b5 (b5). This redox transformation receives electrons via NADPH-cytochrome P450 reductase (CPR), although the protein-protein interactions that regulate these proteins and activities have never been examined directly.

We have used an extremely sensitive balance (~ pico-gram sensitivity) called a quartz crystal microbalance (QCM) to monitor the mass of proteins sequentially added to a chip modified with a membrane bilayer. Each protein binds to the lipid with a unique "signature" profile as the structural impact of the protein on the membrane is recorded. The three protein, CPR:P450c17:cyt.b5, system has been examined comprehensively, introducing each component in different concentrations onto lipid modified chips. Once assembled, the functional activity for each assembly was assessed by introducing the electron donor (NAD(P)H) together with the substrate, pregnenolone, and product (DHEA) was detected using a RIA.

The application of the QCM technique to the assessment of assemblies of proteins on lipid layers will be presented. The rate of association with the surface with/out other protein components has been determined stoichiometrically with remarkable reproducibility. The morphology of proteins associated with the membrane has been revealed using atomic force microscopy enabling visualization of these protein interactions.

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QUANTIFICATION OF MULTIPLE STEROID HORMONES IN MALE MOUSE SERUM AND REPRODUCTIVE TISSUE USING AN LC-MS/MS ASSAY

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LC-MS/MS used to quantify steroid hormones from biological samples has higher specificity than immunoassays and a recent generation of desktop units now has, for the first time, sensitivity matching steroid immunoassay. This study aimed to validate a method developed for human serum samples to measure simultaneously multiple steroids (testosterone (T), dihydrotestosterone (DHT), 3α-androstanediol (3αDiol) and 3β-androstanediol (3βDiol)) in serum (castrate and intact) and steroidogenic (testis) and androgen dependent (prostate) tissues of mice. Tissues were homogenised and steroids were extracted by liquid-liquid extraction (3:2, hexane: ethyl acetate). Reproducibility (repeat injections from pooled serum or homogenate) and linearity (dilution of sample pools) were within acceptable limits using serum and tissue extracts with T measurable in as little as 25µL of male mouse serum. In intact male mice, serum T (6.0 ± 10.2 ng/mL), DHT (1.5 ± 0.6 ng/mL), 3αDiol (0.6 ± 0.7 ng/mL) and 3βDiol (0.7 ±1.1 ng/mL) were readily detectable but T was undetectable in 200 µL serum of orchidectomized mice despite a detection limit of 4 pg on column and excellent recovery of exogenous testosterone spiked into a pool of castrate male serum. T (0.5±0.6ng/g) and DHT (6.1±2.2ng/g) could be measured in all prostate lobes. In addition, 3αDiol was detected in 5/9 and 3βDiol in 2/9 of all anterior prostate samples (detection limits 80 pg on column for both). Steroid quantification from extracts of whole testis was excellent with all target steroids being quantifiable in 8.75 mg of tissue except for DHT (17.5mg). In summary this method allows quantification of multiple steroids (T. DHT, 3a & 3β Diols) from small sample volumes and tissues dictated by the requirement of mouse based research.

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PURIFICATION AND FUNCTION OF RECOMBINANT MURINE GROWTH DIFFERENTIATION FACTOR-9

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GDF-9, a member of the TGF-β superfamily, is an oocyte secreted factor critical for folliculogenesis. Recent studies have also shown the testicular localisation of GDF-9 in a stage-specific manner in round spermatids[1]. The identification of GDF-9 receptors on round spermatids and Sertoli cells suggests that GDF-9 may act as a potential germ cell regulator of Sertoli cell function. Studies utilising GDF-9 have been limited due to the lack of purified protein. Therefore the main purpose of this study was to produce and purify biologically active recombinant murine (rm) GDF-9, and assess its actions upon Sertoli cell functions.

The purification of rmGDF-9 involved a combination of gel filtration chromatography and reversed phase-HPLC. Bioactivity of the purified material was assessed using primary Sertoli cell cultures and a luciferase assay in adrenocortical cells. In initial experiments, GDF-9 was purified to homogeneity; however, its bioactivity was substantially decreased (Bioactivity/ Immunoactivity (B/I) of ~ 0.15). Previous studies[2] had indicated that phosphorylation is important for GDF-9 bioactivity; therefore, we assessed the phosphorylation status of GDF-9 across the purification. Our results indicated that rmGDF-9 produced in HEK293E cells is phosphorylated on threonine and serine residues and that phosphorylation is significantly (80%) decreased following gel filtration chromatography. The inclusion of phosphatase inhibitors blocked the dephosphorylation of GDF-9 and resulted in a significant increase in biological activity.

Purified rmGDF-9 was shown to significantly ($p=<0.001$) up-regulate the expression of inhibin α and inhibin βB subunit mRNA, and significantly down-regulate tight junctions surrounding Sertoli cells. The action of GDF-9 on Sertoli cells suggests a role for this growth factor in spermatogenesis, particularly in regulating the movement of germ cells across the blood-testis barrier and modulating the endocrine feedback role of inhibin.

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MOLECULAR INTERACTIONS THAT GOVERN THE SYNTHESIS, SECRETION AND EXTRACELLULAR MATRIX DEPOSITION OF TGF-B1

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Transforming growth factor β1 (TGF-β1) is secreted as part of an inactive tripartite complex consisting of the mature growth factor, the TGF-β1 prodomain (latency-associated peptide or LAP) and a molecule of latent TGF-β binding protein (LTBP). It is proposed that disruptions in the formation of this complex, or its subsequent interactions with extracellular matrix components, will alter TGF-β1 levels and result in a variety of inflammatory, fibrotic and skeletal disorders. Therefore, we sought to understand the molecular interactions that govern the formation of the large latent TGFβ1 complex. Utilising site-directed mutagenesis, we identified hydrophobic residues (Ile^{53}, Leu^{54}, Leu^{57} and Leu^{59}) in the TGF-β1 prodomain that, when substituted for alanine, were found to be disruptive for TGF-β1 expression. Incorporation of these mutations into TGF-β1 prodomain constructs generated variants that were unable to inhibit the activity of the mature growth factor. Together, these findings indicated that a hydrophobic motif near the N-terminus of the prodomain mediated the assembly, secretion and latency of TGF-β1. Interestingly, further mutagenesis across this region of the prodomain indicated that it was also critical for interactions with LTBP-1. Mutation of key cationic residues (Arg^{45} and Arg^{50}) disrupted the formation of the large latent TGF-β1 complex

and altered the activation status of the secreted growth factor. Finally, the ability of the TGF-β1 prodomain to confer latency to the mature growth factor is dependent upon its capacity to dimerise and we have characterised the dimer interface (Trp^{198}-Ile^{222}) within the TGF-β1 prodomain. This comprehensive analysis of the latent TGF-β1 complex will aid in our understanding of the functional consequence of disease-associated mutations within the prodomains of TGF-β1 and related family members.

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WHAT IS THE SOURCE OF ACTIVIN A RELEASE DURING ACUTE INFLAMMATION? ASSESSMENT OF TISSUE MRNA AND PROTEIN LEVELS FOLLOWING LIPOPOLYSACCHARIDE CHALLENGE

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We have demonstrated previously that activin A is an essential component of the innate immune response (1). Stimulation of mice with the inflammatory agent lipopolysaccharide (LPS) causes activin A in the circulation to increase within about 40 minutes of challenge.The source(s) of this activin A is unclear. We therefore collected 22 different tissues from C57/Bl6 male mice injected with saline alone (n = 5) or injected with LPS one hour previously (n = 5). At this time, circulating concentrations of activin A are at their peak. Tissues were processed for mRNA measurements using real-time RT-PCR (qRT-PCR), or lysates were assayed by activin A ELISA. Relative mRNA expression levels in control mice were highest in the liver, vas deferens, brain cortex, eye, skin, small intestine, adrenal gland and epididymis. Conversely, mRNA levels in the seminal vesicles, thyroid, pancreas and prostate were very low. The effect of LPS on activin subunit mRNA levels was minor, the only significant effect being in the vas deferens where levels declined ($P<0.05$). In terms of tissue concentrations of the protein, bone marrow contained the highest levels of activin A, with significant amounts of activin A also found in skin, adrenal gland, prostate, epididymis and vas deferens. The lowest levels of activin A protein were observed in the eye, lung and testis. LPS treatment did not significantly affect activin A concentrations in most tissues, but caused a 4-fold increase ($P<0.05$) in lung and prostate. We conclude that there are relatively minor changes in activin mRNA expression and protein content at the tissue level following LPS treatment. Consequently, the source of activin A remains unclear, but the initial increase in circulating activin A during acute inflammation may be pre-stored protein released from non-resident leukocytes in the circulation.

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PROSTAGLANDIN TRANSPORTER (HPGT) AND 15-HYDROXYPROSTAGLANDIN DEHYDROGENASE (PGDH) GENE EXPRESSION DECREASES IN A COORDINATE FASHION IN THE HUMAN DECIDUA WITH LABOUR

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Prostaglandins (PGs) are crucial mediators of labour. The balance of PG synthesis and metabolism determines intrauterine PG levels. Hypothetically, decreasing PG-metabolism in the gestational tissues, combined with the well-documented increase of PG biosynthesis, can elevate PG levels to labour-promoting concentrations. Here we determined the expression of key PG metabolic pathway components in the gestational tissues to assess whether decreasing PG-metabolism could contribute to increasing PG levels at labour. Amnion, chorion, decidua and placenta were collected after term elective caesarean section (CS) and spontaneous labour (SL) (n=10-13). mRNAs encoding PGDH (the PG-inactivating enzyme), hPGT (the cellular PG-importer), and MRP4 (performing ATP-dependent extrusion of PGs from cells) were measured by real-time RT-PCR.

These mRNAs were measurable in all tissues. PGDH and PGT mRNA levels were 3 to 20-fold higher in chorion, decidua and placenta than in amnion ($p<0.05$). MRP4 mRNA abundance was 50% higher ($p<0.05$) in decidua than in amnion. Of the two MRP4 mRNA variants, MRP4-1, but not the truncated MRP4-2, was expressed in all tissues. PGT and PGDH mRNA abundance in the decidua was lower after SL than after CS (by 39% and 60%, respectively; $p<0.02$), while there was no change with labour in the chorion and placenta. PGDH and PGT mRNA levels correlated positively in individual deciduas after CS and SL ($p=0.0022$ and 0.0011, respectively). No correlation was detected in the chorion and placenta. MRP4 mRNA abundance did not change with labour.

Thus, (1)PG inactivation in the gestational tissues is a two-step process comprising uptake by hPGT and metabolism by PGDH. (2)PG inactivation is minimal in the amnion. (3)hPGT and PGDH expression is coordinated in the decidua, but not in the chorion and placenta. (4)Decreasing decidual PG metabolism, caused by reduced hPGT and PGDH expression, and PG secretion by MRP4 can contribute to the elevated PG levels promoting labour.

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MULTIVARIATE CLASSIFICATION AND CHARACTERIZATION OF WATER ECOSYSTEMS BASED ON HIGH THROUGHPUT ANALYSIS OF ENDOCRINE DISRUPTING COMPOUNDS USING TEMPERATURE-DEPENDENT INCLUSION CHROMATOGRAPHY

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Quantification of endocrine disrupting compounds (EDCs), particularly steroid hormones in environmental samples, presents unique challenges for samples extraction, concentration and separation. Due to significant differences in compounds polarity and variety of stereo-isomeric forms available, simultaneous quantification of such chemicals in complex mixtures, which are characterised by high level of interfering compounds, is still an unsolved analytical problem. Moreover, an efficient analysis of large data sets typically generated during chromatographic separation and high throughput detection of complex samples is also challenging problem.

This research communication summarize the authors' approach for quantification of wide range of steroids as well as known and unidentified yet low-molecular mass compounds that may act as the endocrine disrupting compounds in water ecosystems [1,2]. Particularly, compounds of interest were selectively isolated from the environmental samples to get a low molecular mass fraction consisting of substances, which polarities ranging from estetrol to progesterone. This was performed using own analytical protocol including optimized solid-phase extraction, separation via temperature-dependent inclusion chromatography and UV-Vis-DAD detection. Quantitative data derived from recorded chromatographic profiles and selected physicochemical measurements (pH, O2, T) were inspected with the multivariate statistical procedures involving agglomerative hierarchical clustering (CA) and principal components analysis (PCA).

Using described above analytical approach the samples derived from different water ecosystems including Baltic Sea, selected lakes and rivers of the Middle Pomerania in northern part of Poland as well as untreated and treated sewage water from municipal sewage treatment plant near Koszalin were analyzed and their EDCs profiles collected. Moreover, some preliminary data concerning selected steroids biodegradation under activated sludge conditions were also investigated. CA and PCA analysis of the acquired data sets confirms a high separation and quantification throughput of the SPE and isocratic HPLC protocols proposed, toward simple and rapid classification of the environmental samples characterized by different sources of EDCs loading.

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THYROID FUNCTION TESTS DO NOT IMPROVE THE PREDICTIVE VALUE OF FIRST TRIMESTER SCREENING FOR PRETERM DELIVERY

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Introduction: Low multiple of medians (MoM) of Pregnancy-Associated Plasma Protein-A (PAPP-A) in first trimester screening are associated with chromosomal abnormalities, birth defects, preterm births and pregnancy loss. Previous studies have shown that thyroid autoimmunity as determined by positive thyroid antibodies is associated with miscarriages and premature delivery.

Aim: To assess the predictive value of thyroid function tests and biochemical markers of first trimester screening, such as PAPP-A, for preterm delivery (defined as ≤37 weeks gestation).

Method: A cohort of 2583 pregnant women attending Western Diagnostic Pathology from October 2006 to March 2007 were recruited for first-trimester specific thyroid function reference interval study. Subsequent deliveries and adverse pregnancy and foetal events were obtained from the Western Australia midwife and birth registries.

Results: There were 2486 births obtained from the registries and pregnancy outcome was available in 2419 women after exclusion of 28 women with twin pregnancies or incomplete data.

Data given as mean (SD) or number (%):

	Gestation		p-value
	<37 weeks (n=148)	≥37 weeks (n=2271)	
Maternal Age (years)	30.5 (5.6)	31.0 (5.2)	0.313#
Smoker	25 (17%)	213 (9%)	*0.006**
Positive Thyroid Antibodies	17 (11%)	355 (16%)	0.196*
TSH (mU/L)	0.98 (0.87)	0.98 (1.2)	0.855#
FT4 (pmol/L)	13.6 (2.3)	13.7 (2.1)	0.357#
FT3 (pmol/L)	4.5 (1.0)	4.4 (2.1)	0.192#
PAPP-A (MoM)	1.08 (0.69)	1.42 (0.96)	*<0.001*#

*Fisher's Exact Test #Mann-Whitney test, mean (SD)

Conclusion: Preterm delivery is associated with low PAPP-A MoM but not with thyroid autoimmunity or thyroid function.

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REVERSIBLE PANCYTOPENIA WITH TREATMENT OF THYROTOXICOSIS DUE TO GRAVES' DISEASE

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Prolonged untreated Graves' disease associated with pancytopenia is an uncommon presentation. Single lineage cytopenias such as isolated leucopenia are frequently seen in Graves' disease, and agranulocytosis can be a serious complication of thionamide use. A full blood examination is prudent prior to treatment with thionamides as severe pancytopenia may require treatment with immunosuppressive therapy or granulocyte-colony-stimulating factor[1]. We present a case report of a 39-year-old female who had pancytopenia on initial diagnosis of severe thyrotoxicosis due to Graves' disease. Initial investigations are summarized below.

		Reference range
TSH	<0.01 mU/L	0.35-5.60
Free T4	72.7 pmol/L	7.5-21.1
Free T3	34.9 pmol/L	3.8-6.0
Haemoglobin	103 g/L	115-165
White cell count	2.5 $x10^9$/L	4.0-11.0
Neutrophil count	1.1 $x10^9$/L	2.0-7.5
Platelet count	79 $x10^9$/L	150-440
TSH receptor antibody	>40	<1.22
Antineutrophil antibody(ANA)	1:320 speckled pattern	

There were no diagnostic features on blood film and haemolysis screen, B12, Folate, viral serology, extracted nuclear antigens and double-stranded DNA antibody were normal. Treatment with carbimazole resulted in gradual improvement of symptoms and normalization of cytopenias 2 weeks after commencing treatment. Bone marrow biopsy was not performed.

There have been few cases of pancytopenia associated with untreated Graves' disease however isolated leucopenia is not uncommon1,2,3. Various mechanisms have been postulated; decreased neutrophil circulation time4, reduced marrow granulocyte reserve5 or an autoimmune or direct toxic mechanism. TSH receptor antibodies may also cross react with thyrotropin-binding moieties on neutrophils6. ANA is elevated in approximately 50% of patients and may play a role in binding neutrophils6. These mechanisms however do not explain pancytopenia.

Reversible myelodysplastic syndrome has been described in one patient with hyperthyroidism7 and it has also been suggested that excess thyroid hormone may affect the maturation and differentiation of the pluripotential stem cell2. This seems most plausible in explaining pancytopenia and the reversible suppression of all lineages of haematopoietic cells in our patient supports this hypothesis.

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ACCESS AND IMMULITE THYROGLOBULIN ASSAY DIFFERENCES IN THE PRESENCE OF ANTI-THYROGLOBULIN ANTIBODIES

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Circulating TgAb can interfere in Tg immunoassays. Interference is variable between assays, is not necessarily related to TgAb level and generally causes false lowering in immunometric methods [1]. This report compares Tg measurements by the Access and Immulite assays in specimens negative and positive for Tg antibodies.

Methods: Tg was measured by immunochemiluminometric assay (ICMA) on Access2 (Beckman-Coulter) and Immulite (Siemens) analysers in specimens (n=137) exchanged between Endolab (Christchurch) and LabPlus (Auckland) and from the Waikato Laboratory QC program (one Access, two Immulite platforms). Ten samples in the 2009 RCPA QAP Tumour Marker Program were also tested. TgAb was measured using Access methodology and values >2.2 IU/ml regarded as positive. Tg results from Immulite and Access assays were compared in TgAb-positive and negative specimens by linear regression and by calculation of Immulite Tg as a percentage of Access Tg concentration.

Results: In TgAb-negative samples, Immulite Tg concentrations correlated (r=0.999) and were higher than Access measurements (Deming regression slope=1.213(1.131-1.294), intercept=-0.05, n=75). In the presence of TgAb some samples showed lower Tg by Immulite than Access, r=0.974, Deming slope= 0.727(0.586-0.869), intercept=0.32, n=62. Log-log plots showed greater dispersion around the regression line for TgAb-positive than negative samples (F=7.2; p<0.001). In Waikato QC, mean Immulite Tg was 113±29 percent of Access Tg in 18

TgAb-negative samples and 55±29 percent in 22 TgAb-positive samples (means±SD). In 10 RCPA QAP samples, Access Tg results were higher than for other ICMA (median for all ICMA was 53±10 percent of Access Tg median) possibly because of TgAb presence in all samples (14±1.6 mIU/L).

Conclusion: There is good correlation in Tg measurement by Access and Immulite assays in TgAb-negative samples however the assays are differently affected by TgAb presence with potential diagnostic consequences. Tg method comparisons in Quality Assurance Programs are compromised when TgAb-positive material is used.

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PSYCHOLOGICAL DISTRESS DECREASES FROM HIGH TO NORMAL LEVELS OVER TWELVE MONTHS IN GRAVES' DISEASE

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Background: Studies of the presence and course of psychological distress (PD) in Graves' disease (GD) have been inconsistent.

Methods: Prospective study of 60 women (age > 18yr, non-pregnant, no previous history of GD) diagnosed with GD and managed by two physicians experienced in disorders of the thyroid. Treatment comprised 12 months antithyroid medication. Levels of PD were measured using the Hospital Anxiety and Depression Scale (HADS) questionnaire. PD was defined as a HADS score greater than 19. The questionnaire was administered at or around the time of first consultation and when antithyroid medication was withdrawn. Other variables recorded included the presence or absence of preceding stressful life events and the levels of thyroid hormones.

Results: During the 12 months of medical therapy for hyperthyroidism, a significant decrease occurred in the mean HADS score (16.7 ± 7.4 vs. 9.8 ± 6.3; P=0.001), and the respective prevalence of PD (36.7% vs. 12.5%; P=0.02). The twelve month values are similar to those found in a non-clinical sample. There was no relationship between PD and preceding stressful life events nor between PD and the level of thyroid hormones.

Conclusions: The results of this study support those studies that show that women presenting with GD have a significantly increased prevalence of PD, which normalises after antithyroid treatment.

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PRECEDING STRESSFUL LIFE EVENTS AND SUBSEQUENT REMISSION IN GRAVES' DISEASE

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Background: Graves' disease (GD) has been shown to be precipitated by stressful life events (SLEs) and to be associated with psychological morbidity. This study concerned the possible relationship suggested by a previous small study between preceding SLEs and subsequent remission in GD.

Methods: Prospective study of 60 women (age > 18yr, non-pregnant, no previous history of GD) diagnosed with GD and managed by two physicians experienced in disorders of the thyroid. Treatment comprised 12 months antithyroid medication, with remission assessed at 18 months. The presence of preceding SLEs was assessed prospectively by both a Life Changes questionnaire (LCQ) and by an unstructured direct physician-patient interview. Goitre size, the levels of thyroid hormones and age were considered as confounding factors. Psychological distress was measured by the Hospital Anxiety and Depression Scale questionnaire (HADS score). Remission was defined as biochemical euthyroidism 6 months after discontinuation of antithyroid therapy.

Results: The overall remission rate was 62%. As expected, remission was significantly predicted by both goitre size and baseline thyroid hormone levels. There was no relationship between the presence of a prior SLE as defined by LCQ and subsequent remission. There was a positive relationship of borderline significance between the presence of physician -defined SLE and subsequent remission . The presence of physician defined SLE was independent of other variables. There was no relationship between HADS score and remission.

Conclusions: Any relationship between prior SLEs and subsequent remission in Graves' disease remains unproven and our positive findings with regards subjective assessment remain suggestive only. Further studies with different methodologies may clarify this question further.

Table 1: Significance of baseline study variables in the outcome of Graves' disease

	Remission	No-Remission	P
Age (years)	37/60a 43.8 ± 12.0	40.6 ± 9.8	0.17
FT3 (nmol/L)	31/53 13.2 ± 8.1	25.7 ± 14.1	0.001
FT4 (nmol/L)	36/59 35.2 ± 17.4	58.3 ± 26.9	0.001
Goitre (large)	14/52 9.4 %	55%	0.001
HADS (total)	31/49 16.5 ± 7.3	17.0 ± 7.7	0.82
SLE (subj.) c	38/60 73.0 %	47.8 %	0.0046b
SLE (LCQ)	41/50 81.1%	83.3%	0.84

a number patients achieving remission/ total sample size

b probability that odds-ratio is greater than 1

c subj.- SLE as per subjective physician assessment

Table 2: Differences in variables between GD patients with and without a physician judged SLE

	SLE-GD	non-SLE GD	P
Number	38	22	
Age (years)	40.8 ± 12.4	45.6 ± 8.2	0.11
FT3 (nmol/L)	17.9 ± 14.4	19.1 ± 8.9	0.73
FT4 (nmol/L)	43.1 ± 24.2	46.1 ± 24.9	0.65
Goitre (large)	27.3 %	26.3 %	0.94
HADS (total)	17.7 ± 6.7	14.7 ± 8.3	0.18
Remission	71.1 %	45.5 %	0.046a

a -probability that odds-ratio is greater than 1

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ACCURACY OF PRE-OPERATIVE LOCALISATION OF PARATHYROID ADENOMA USING 99MTC-PERTECHNETATE SESTAMIBI SCAN AND ULTRASOUND IN PATIENTS WITH PRIMARY HYPERPARATHYROIDISM UNDERGOING PARATHYROIDECTOMY

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Background: A unilateral approach to parathyroidectomy, with removal of an adenoma and identification of an ipsilateral normal gland, has potential advantages of shorter operative time, less scarring and less post-operative hypocalcaemia [1] . Minimally invasive parathyroidectomy (MIP) is a further refinement, with surgery limited to removal of the adenoma via a small (<4cm) incision. MIP is increasingly performed under local anaesthetic, resulting in lower complication rates and lower morbidity. [2] These newer techniques are critically dependent on the ability to accurately identify a solitary adenoma pre-operatively.

From 2005, all patients referred for parathyroidectomy from an endocrinology practice underwent a parathyroid sestamibi scan and a parathyroid ultrasound performed by a single operator.

Study design: Retrospective clinical audit of all patients undergoing parathyroidectomy between April 1st 2005 and March 31st 2009 referred by a single endocrinologist. The accuracy of pre-operative imaging (sestamibi and ultrasound) in correctly localising the adenoma was assessed.

Results: 111 patients who underwent parathyroidectomy for primary hyperparathyroidism patients were identified via searching of electronic medical records. 71% were female (F=79, M=32). Mean age was 61.4+/-10.8 years. 58% had mild (Ca-c 2.5-2.75), 35% had moderate (Ca-c 2.76-3.0) and 7% had severe disease (Ca-c>3.01).

The sensitivity of pre-operative ultrasound for correctly localising the site of adenoma found at surgery was 68%. Sensitivity was lowest in the first 12 months at 52%, then rose to 68-75% in the following three years suggesting that operator skill improved with experience. This compared to a sensitivity of parathyroid sestamibi of 71%.

The rate of minimally invasive parathyroidectomy rose from 11% in the first three years, to 48% in the last year.

Conclusions: Preoperative imaging with a combination of ultrasound and sestamibi correctly indentifies the location of the adenoma in greater than two thirds of patients. Accurate localisation enables increased utilisation of a localised or minimally invasive parathyroidectomy.

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THE ASSOCIATION OF ANTI-THYROGLOBULIN ANTIBODY WITH INCREASED CANCER RISK IN THYROID NODULES

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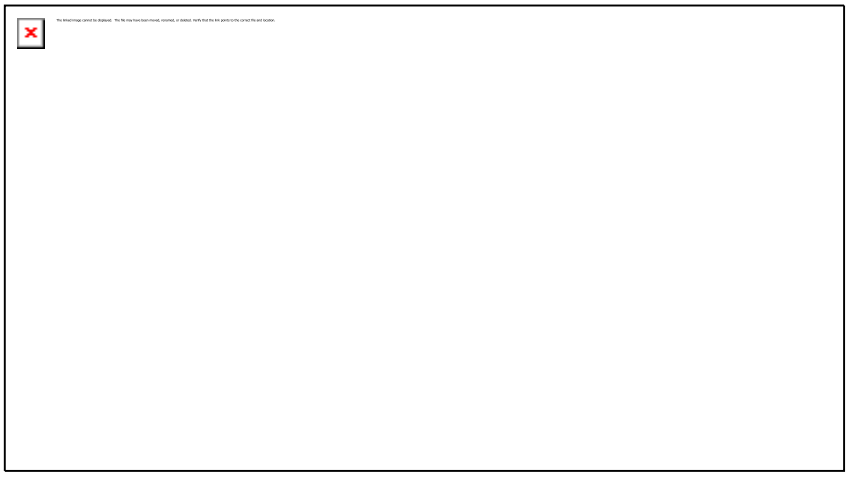
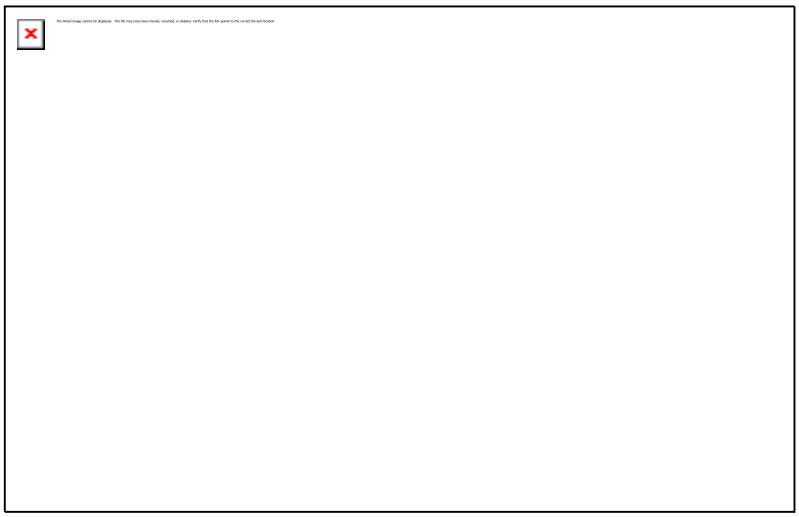
Background: The association between autoimmune thyroiditis and thyroid cancer is still not clear. However, recent reports presenting TSH as a potential marker for thyroid cancer suggest the possible association between thyroid malignancy and autoimmune thyroiditis through elevation of TSH levels. The purpose of this paper is to investigate whether thyroid cancer was associated with thyroid autoimmunity in thyroid nodules.

Method: We reviewed the records of patients with thyroid nodules evaluated by ultrasonography guided-fine needle aspiration cytology at our institution from 2006 to 2008. The cytology results were classified into inadequate, benign, indeterminate, and suspicious or frank malignancy. Thyroid autoimmunity was assessed by measuring thyroglobulin antibody (TgAb) and thyroperoxidase antibody (TPOAb) in addition to thyroid function test. The final outcome was designed combining cytology and histology agreeably.

Results: Of the 1638 patients, the positive rates of TPOAb and TgAb were 18.7 %, 15.2%, respectively. Among operated 238 patients, 204 had papillary thyroid cancer. Malignant thyroid nodules had higher prevalence of positive TPOAb (not TgAb), also features of having decreased age, size and non solitary ones (Table 1).



In Multivariate logistic analysis, increased thyroid cancer had significant association with positive TgAb (OR 1.58, CI 1.02-2.46) in addition to elevated TSH, age, nodule size(Table 2).



Conclusion: Positive TgAb was an independent predictor for thyroid cancer besides TSH and clinical risk factors. These data suggest more attention and frequent follow-up is needed for the thyroid nodules of patients with positive TgAb

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COMPLIANCE WITH THYROID FUNCTION MONITORING IN PATIENTS ON AMIODARONE

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Amiodarone is a well established treatment for the management of supraventricular and ventricular arrhythmias. Its use is associated with both hyperthyroidism and hypothyroidism. Guidelines have been developed for appropriate monitoring of thyroid function (TFTs) in patients on amiodarone (Goldschlager 2000). The aim was to determine the cross-sectional number of patients on oral amiodarone in Liverpool Hospital; the indication for use of oral amiodarone; the coincidental presence of thyroid disease and whether any assessment of thyroid function was performed during or within six months prior to their admission.

A cross sectional audit based on review of records of adult patients admitted over a one month period who had been identified by nursing and pharmacy staff to be on oral amiodarone was performed.

Over one month 41 patients received oral amiodarone during their hospital admission. Mean age was 73 years (range 48-89), and 17 were female. The most frequent indication was atrial fibrillation/flutter. 16 patients had amiodarone initiated during their admission. Of these, only 9 had baseline thyroid function studies performed. Only 14 of those admitted on amiodarone had an assessment of TFTs. Of those admitted on amiodarone, 6 were also receiving thyroxine. One patient was receiving carbimazole on admission, commenced on amiodarone during her admission but an endocrine consultation was not sought.

Assessment of TFTs in those receiving amiodarone was suboptimal. This may be due to a lack of awareness of the need for TFT monitoring in those on amiodarone. This is of importance since significant thyroid dysfunction may worsen the initial indication for amiodarone.

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A FOUR YEAR AUDIT OF THYROID CYTOLOGY FROM A SINGLE ENDOCRINOLOGISTS' PRACTICE

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Aim: To audit the accuracy of thyroid cytology against histopathological diagnosis to ensure best possible care of patients.

Methods: An electronic database of a single endocrinologist's (SM) patient's files was examined for all thyroid cytology performed between 1/1/04 and 31/12/08. The subsequent histopathology from all non-benign cytology specimens was reviewed.

Results: 597 cytology episodes were recorded in the period and of these 61 (10.2%) were reported as non-benign (See table). There was no histopathological comparison on 8 patients as they did not have surgery for various reasons eg patient choice, operative risk too high. One of these clinically had anaplastic thyroid cancer and died within 3 weeks of presentation. Of the remainder 25 (46%) had malignant diagnoses. All patients classified as papillary lesion, suspicious for or clearly papillary carcinoma on cytology had papillary carcinoma. Only 10 (26%) cytologies designated as atypical, follicular lesion or follicular neoplasm had a malignant diagnosis (See table).

Conclusion: There remain significant limitations in the preoperative cytological diagnosis of thyroid nodules particularly in the group labelled as atypical or follicular lesions. Work is progressing in our team to improve communication between clinician and pathologist.

Cytological Diagnosis	#	Pathological Diagnosis	#
Atypical	36	MNG with hyperplastic nodule	4
		Follicular adenoma	13
		Atypical follicular adenoma	1
		Hurthle cell adenoma	3
		Follicular carcinoma	3
		Follicular variant papillary carcinoma	2
		Papillary carcinoma	3
		Metastatic renal cell cancer	1
		No surgery	6
Follicular lesion	5	Follicular adenoma	3
		Hurthle cell adenoma	1
		No surgery	1
Follicular neoplasm	5	Follicular adenoma	3
		Follicular carcinoma	1
		MNG with hyperplastic nodule	1
Papillary lesion	1	Papillary carcinoma	1
Papillary carcinoma	11	Papillary carcinoma	11
Other eg possible medullary thyroid carcinoma, carcinoma, high grade malignancy	3	Follicular variant papillary carcinoma	1
		Metastatic non small cell (adenosquamous) carcinoma	1
		Presumed anaplastic thyroid carcinoma	1

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THYROID CANCER IN GRAVES' DISEASE: AN UPDATE ON EVALUATION AND MANAGEMENT

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Controversies still exist regarding the association between thyroid cancer and Graves' disease. The literature on the prevalence and aggressiveness of thyroid cancer in Graves' disease is not consistent. There are no evidence based guidelines on the evaluation of thyroid nodules in Graves' disease. Therefore most centres are basing best practice on local experience.

We report a case of a 45 year old woman with longstanding Graves' disease who was euthyroid on Carbimazole, and presented with a palpable small right thyroid nodule and cervical lymphadenopathy. Ultrasound-guided fine needle aspirate biopsy (FNAB) revealed papillary thyroid cancer with extensive locoregional involvement. She underwent total thyroidectomy with lymph node clearance followed by ^{131}I ablation.

We conducted a literature review through PubMed and Medline (1950 – current) to examine the assessment and management of thyroid cancer associated with Graves' disease. There is no consistent data to demonstrate a higher prevalence of thyroid nodules (33.6%) and thyroid cancer (0 – 12%) in Graves' disease compared to the general population (nodules: 30 – 50%; cancer: 2.3 – 2.7%). Recent studies have been based on retrospective observation of surgical cases from various geographical areas and genetic backgrounds. Several earlier surgical series and 2 retrospective case-control studies suggested more aggressive thyroid cancers (nodal and extracapsular involvement, and distant metastasis) in Graves' disease compared to euthyroid controls. One recent retrospective case-control study however has not shown a worse outcome in the Graves' disease group.

There is paucity of evidence in the literature to guide recommendations for routine screening for thyroid nodules in patients with Graves' disease. Ultrasound imaging and FNAB may be appropriate in patients with palpable or cold nodules especially in the older age group (≥45) due to higher malignant risk. Prospective case-control studies will be necessary to elucidate the true nature and clinical course of thyroid nodules and cancer in Graves' disease.

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RISK FACTORS FOR HYPOTHYROIDISM: A 13 YEAR, LONGITUDINAL ANALYSIS OF A COMMUNITY-BASED COHORT USING CURRENT ASSAY METHODOLOGY

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Background. There are few long term studies of the natural history of autoimmune hypothyroidism. In previous studies, positive thyroid antibodies measured by semiquantitative methods were a strong predictor of hypothyroidism, with similar predictive value to raised TSH. This has not been confirmed for antibodies measured by current, automated immunoassay techniques.

Methods. We measured TSH, free T4, thyroid peroxidase antibodies (TPOAb) and thyroglobulin antibodies (TgAb) using the IMMULITE platform on archived sera from 1184 subjects (611 females) who participated in the 1981 and 1994 Busselton Health Surveys. Subjects who were on thyroxine treatment, were hyperthyroid or had untreated overt hypothyroidism at baseline were excluded. Outcome measures of hypothyroidism were HYPO1, defined as serum TSH above 4.0 mU/L or on thyroxine treatment and HYPO2, defined as TSH above 10.0 mU/L or on thyroxine treatment.

Results. At 13 years follow-up, 110 subjects (84 women) had HYPO1 and 42 (38 women) had HYPO2. In multivariate regression analysis, TSH, female sex, TPOAb and TgAb were significant predictors of hypothyroidism. A receiver operator curve suggested that baseline TSH above 2.5 mU/L was the optimal cut-off for predicting HYPO1, with sensitivity 73% and specificity 91%. Of 76 antibody-positive subjects with baseline TSH 2.5 mU/L or less at baseline, 6 developed HYPO1 (7.9%; 95% CI 1.8-14), and 1 developed HYPO2 (1.3%; 95% CI 0.0-3.9).

Conclusions. Thyroid antibodies measured by immunoassay are a significant predictor of hypothyroidism. However, the absolute risk of developing hypothyroidism in unequivocally euthyroid, antibody-positive subjects with TSH 2.5 mU/L or less is small, approximating 0.1-0.6% per year.

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