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ESA OFFICE BEARERS 2006

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PAST ESA OFFICE BEARERS 1958-2006

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 C.W.Emmens (March 63)		I.Thomas	I.Thomas
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. Zajac	L. Bach	M. McLean	V. Clifton

ESA INTERNATIONAL TRAVEL GRANT

2003 Emma Ball

2004 Gordon Howarth
Sophie Chan
Vincenzo Russo

2005 Stuart Ellem

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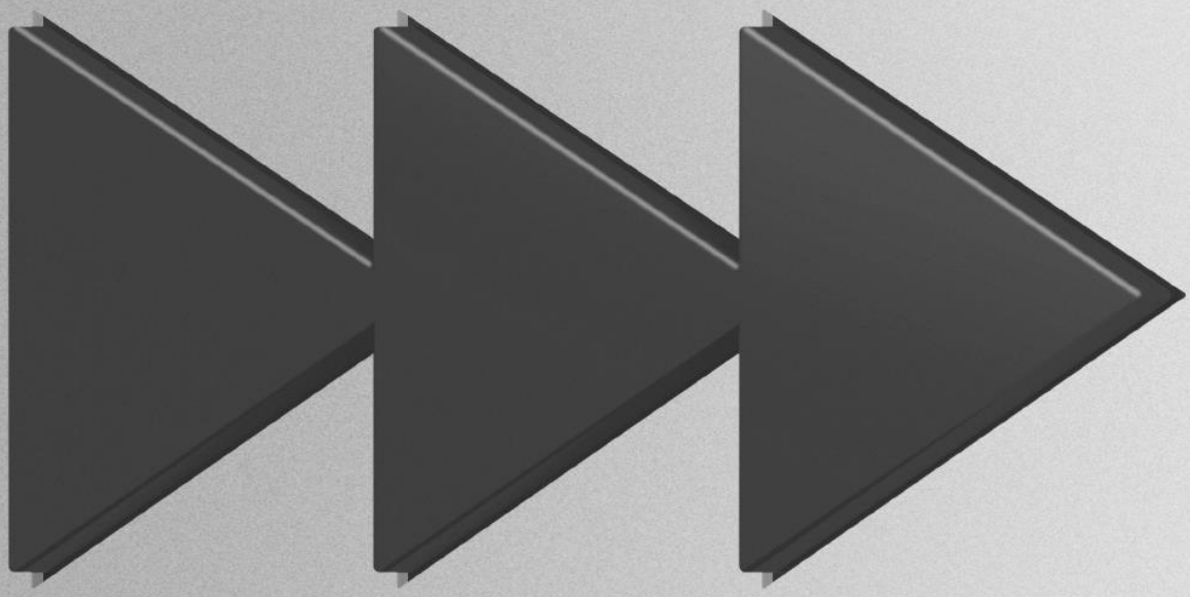
FUTURE MEETINGS

2007

ESA Seminar 2007
4th - 6thMay 2007
Sebel Lodge Yarra Valley, VIC
www.esaseminar.org.au

ESA Clinical Weekend 2007
31st August – 2nd September 2007
New Zealand
www.esaclinicalweekend.org.au

Combined ESA/SRB Annual Scientific Meeting
2ND – 5TH September 2007
Christchurch Convention Centre, NZ
www.esa-srb.org.au



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Avandia®
rosiglitazone maleate

*PBS changes including Triple Therapy have simplified the Avandia prescribing requirements compared to the original listing.

PBS Information: Authority Required. Refer to PBS Schedule for full Authority Required information.

Please review Product Information before prescribing. AVANDIA (rosiglitazone maleate). Indications: Treatment of Type 2 diabetes mellitus. Monotherapy, dual therapy with sulphonylureas, metformin or insulin and in triple therapy with metformin and sulphonylureas, in patients inadequately controlled by diet and exercise. **Contraindications:** Hypersensitivity. Precautions Type 1 diabetes mellitus, premenopausal anovulatory patients with insulin resistance (eg. polycystic ovary syndrome), New York Heart Association (NYHA) classified heart failure, hepatic dysfunction/impairment, pregnancy (Category B3), lactation, children. **Interactions:** Gemfibrozil, rifampicin. **Adverse Events:** headache, back pain, hyperglycaemia, fatigue, diarrhoea, hypoglycaemia, oedema, anaemia, hypercholesterolaemia, weight gain, hepatic dysfunction - This is not a full list, for more details refer to full PI. **Dosage:** Initiated at 4mg/day. Can be increased to 8mg/day after 6-8 weeks if greater glycaemic control is required. May be given once or twice daily. Careful dose titration when adding to insulin therapy. Full Disclosure Product Information is available from GlaxoSmithKline Australia Pty Ltd, 1061 Mountain Highway, Boronia, Vic 3155. ABN 47 100 162 481. Avandia® is a registered trade mark of the GlaxoSmithKline Group of Companies. **PBS dispensed price (authority required):** Avandia 4mg $61.70 for 28 tablets (5 rpts); Avandia 8mg $92.34 for 28 tablets (5 rpts) GSKA PC060138 2'06.

KEITH HARRISON MEMORIAL LECTURES

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

NOVARTIS JUNIOR 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

ESA MAYNE PHARMA BRYAN HUDSON CLINICAL ENDOCRINOLOGY AWARD

The ESA Mayne Pharma 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 Davidson
2005	Carolyn Allan

SCHERING

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testosterone undecanoate

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Sustained testosterone management

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Reandron® 1000, solution for injection: testosterone undecanoate 1000mg/4ml. **Indication:** testosterone replacement in primary and secondary male hypogonadism, where testosterone deficiency has been confirmed by clinical features and biochemical tests. The recommended dose for adult men is 1 ampoule injected intramuscularly every 10 to 14 weeks into the gluteal muscle. Special care must be taken to avoid intravenous injection and injections must not be given subcutaneously. The first injection interval may be reduced to a minimum of 6 weeks to achieve steady-state testosterone levels more rapidly. **Contraindications:** Prostate/breast carcinoma, use in women/children, hypercalcaemia accompanying malignant tumours, hypersensitivity to testosterone undecanoate or the excipients. **Precautions:** Regular prostate and haemoglobin/haematocrit monitoring. Patients with severe cardiac/hepatic/renal insufficiency, hypertension, epilepsy or migraine, bleeding or coagulation disorder. Risk of sleep apnoea. Potential interaction with oral anticoagulants, corticosteroids, oxyphenbutazone, cyclosporin. Decreased insulin requirement. **Adverse effects:** in addition to those already noted: diarrhoea, leg pain, arthralgia, dizziness, increased sweating, headache, respiratory disorder, acne, breast pain, gynaecomastia, pruritus, skin disorder, testicular pain, injection site pain, subcutaneous haematoma at the injection site.

Please review Approved Product Information before prescribing. TGA approval date: 24.10.05. Approved Product Information is available from Schering Pty Limited, ABN 50 000 023 361, Locked Bag 4, Alexandria, NSW 1435. Distributors for Schering AG, Germany. ®Registered Trademark. AU2006.0131 A54 07.06

Andrology

PBS Information: Authority Required. Refer to PBS Schedule for full information.

SC768

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
2005	Renea Taylor

HONORARY LIFE MEMBERS

Prof 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 De Krester
Prof C.J. Eastman AM
Dr K.A. Ferguson
Prof John W. Funder
Prof R.D. Gordon
Dr Ian B. Hales
Dr Philip Harding
Prof Basil Hetzel
Dr Brian Hirschfeld
Dr Ivan G. Jarrett
A/Prof Stephen Judd
Prof Richard G. Larkins
Prof Leslie Lazarus
Dr T.B. Lynch
Prof T. John Martin
Dr Len Martin
Dr F.I.R Martin
Dr Ian C.A. Martin
Prof Solomon Posen
Prof Marilyn Renfree
Prof T.J. Robinson
Prof Alfred W. Steinbeck
Prof Jim Stockigt
Dr Ian D. Thomas
Emeritus Prof John R. Turtle
Dr A.L. Wallace
Prof Marelyn Wintour-Coghlan
Dr K.N. Wynne

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The Local Organising Committee
SRB: Grant Montgomery (Chair), Jean Fleming, Marie Pantaleon
ESA: Judith Clements

SRB Program Organising Committee
Sarah Robertson, Eileen McLaughlin, Darryl Russell, Ann Drummond, Sarah Meacham & Claire Roberts

ESA Program Organising Committee
Stephen Twigg (Chair), Anne Nelson, Charles Allan, Roderick Clifton-Bligh, Shaun McGrath & Paul Williams

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

Society Secretariat
Endocrine Society of Australia (ESA)
Ivone Johnson
145 Macquarie Street
SYDNEY NSW 2000
Ph: 02 9256 5405 Fax: 02 9251 8174
Email: esa@racp.edu.au
Website: www.endocrinesociety.org.au

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AWARDS

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Research Centre for Reproductive Health

Reproduction, Fertility and Development

PRINCIPAL GUEST SPEAKERS FOR 2006

Eiichi Araki
Professor Araki is Professor and Director of the Department of Metabolic Medicine, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University. His honours and awards include the 1989 Kato Memorial Young Investigator's Research Award, 1990 Young Investigator's Award of the Japanese Society of Internal Medicine, 1994 Mary K. Iacocca Research Fellow (honorary fellow), 1998 Shionogi-Lilly Award, Japan Diabetes Society and the 1999 Young Investigator's Award, Kumamoto Medical Society.

William Crowley M.D.
Dr. William Crowley is a Professor of Medicine at Harvard Medical School, Director of the Harvard Reproductive Endocrine Sciences Center, Chief of the Reproductive Endocrine Unit and Director of Clinical Research at the Massachusetts General Hospital (MGH). He graduated from Holy Cross College in 1965, Tufts Medical School in 1969, and has been at the MGH since 1969. Dr. Crowley is an active clinical investigator whose main research interest is discovering the genes that control reproduction in the human using various disease models of both sexes. He also directs a Training Program for clinical and basic investigators in Developmental Biology and Reproductive Endocrinology and has an active interest in the national program of clinical research in academic health science centers. He is the Founder and current President of the Clinical Research Forum, an association of 48 of the US's leading academic medical centers involved in human research. He is also the past President of the Endocrine Society, the recipient of the 2005 Fred Conrad Koch Award (The Endocrine Society's highest scientific award), The Mentor of the Year Award from Women in Endocrinology (first male recipient), a former Board Member and Executive Committee member of FASEB, and a former member of the Institute of Medicine's Clinical Research Roundtable.

Creswell J Eastman
Endocrinologist and former President of Endocrine Society of Australia, Vice President Asia Oceania Thyroid Associatio, Chairman of ACCIDD and Vice Chairman of Board of International Council for Control of Iodine Deficiency Disorders (ICCIDDD)

Sadaf Farooqi
Dr I. Sadaf Farooqi qualified with honours in Medicine from the University of Birmingham in 1993 and was awarded the Queen's Scholarship (gold medal) for best overall academic performance. After junior hospital posts in Birmingham and Oxford she moved to Addenbrooke's Hospital, Cambridge, as part of a Wellcome Trust Training Fellowship. During this time of her PhD, she identified the first single gene defect to cause human obesity in two children with a mutation in the gene encoding the hormone leptin, published in Nature in 1997 and New England Journal of Medicine in 1999. Since the award of a Wellcome Trust Clinician Scientist Fellowship in 2002, Sadaf has continued studying the genetic basis of severe human obesity at the Dept of Clinical Biochemistry, Cambridge, in parallel with her clinical training in General Medicine, Diabetes and Endocrinology. She has been invited to speak at numerous International research meetings and has recently described mutations in other genes such as MC4, WNT10B and TrkB, which may have functional consequences for cases of human obesity.

Peter Leedman
Peter Leedman graduated in medicine (UWA) and completed training in endocrinology and his PhD (WEHI) before a post-doc at Harvard Medical School with Bill Chin. He returned to Perth in 1994 to take up an academic position in medicine at UWA based at Royal Perth Hospital. Since then, he has been recognized internationally for his work on hormone action, in particular the mechanisms regulating expression of key targets for therapeutics in hormone-dependent cancer (including the androgen and growth factor receptors). More recently, the discovery of several novel nuclear receptor coregulators that modulate estrogen and androgen signaling, has provided a range of new potential biomarkers and therapeutic targets. He has received several awards, including the UK Endocrine Society Asian and Oceanic Medallist in 2004. He currently serves on the editorial boards of the Journal of Molecular Endocrinology and Endocrine Reviews and is Deputy Director of the Western Australian Institute for Medical Research (WAIMR) in Perth..

Marilyn Renfree
Marilyn's primary research interest is the developmental biology, reproductive physiology and endocrinology of mammals. Her research has been almost all on marsupials, because their unique reproductive strategy provides unrivalled opportunities to understand these processes. Marsupials give birth to small underdeveloped young that undergo the majority of their development external to their mother in the pouch, making it possible to study the control of normal organ growth throughout developmental stages otherwise inaccessible in utero. Her laboratory is known internationally for its innovative studies of these unique and charismatic Australian animals. Marilyn is a Laureate Professor of the University of Melbourne and an ARC Federation Fellow.

Manuel Tena-Sempere
M. Tena-Sempere is presently holding a position as Associate Professor in Physiology at the University of Cordoba, Spain. His expertise lies within the field of Reproductive Biology and Neuroendocrinology. His main areas of interest within Reproductive Endocrinology include: (1) Analysis of the neuroendocrine networks controling gonadotropin secretion, with special attention to kisspeptin physiology; (2) Characterization of the molecular mechanisms and physiological signals responsible for the intergrated control of reproduction, energy balance and metabolism; (3) Identification of novel markers and molecular mechanisms for endocrine disruption of the reproductive axis at the hypothalamic-pituitary level; and (4) Study of gonadal physiology, with special attention to the testis. In the last 12 years, M. Tena-Sempere has published over 100 articles in international peer-reviewed journals on the topics indicated above.

Bill Thatcher

William W. Thatcher is a Graduate Research Professor Emeritus in the Department of Animal Sciences at the University of Florida. He received his B.S. from the University of Maryland, a M.S. degree from the University of Maryland, in conjunction with the USDA-ARS Beltsville Research Center, and the Ph.D. degree from Michigan State University. He completed two sabbaticals at INRA, Nouzilly, France, in 1977 and 1985. His research program in cattle has been associated with ovarian follicular development, maternal-embryo interactions, and developmental approaches for regulating reproductive function to enhance production and health. He has served as a mentor for 70 graduate students-postdoctoral fellows and sabbatical persons. Dr. Thatcher has published 311 refereed journal articles and 40 book chapters (http://www.thatcherteam.com).

INFORMATION FOR DELEGATES & PRESENTERS

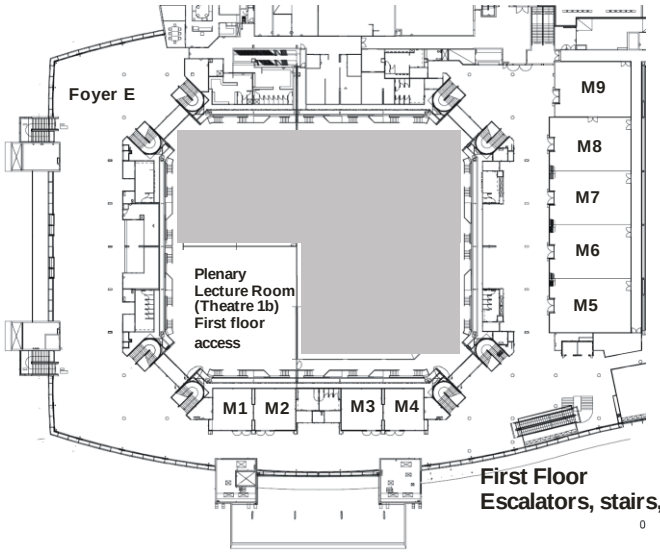
Venue Location

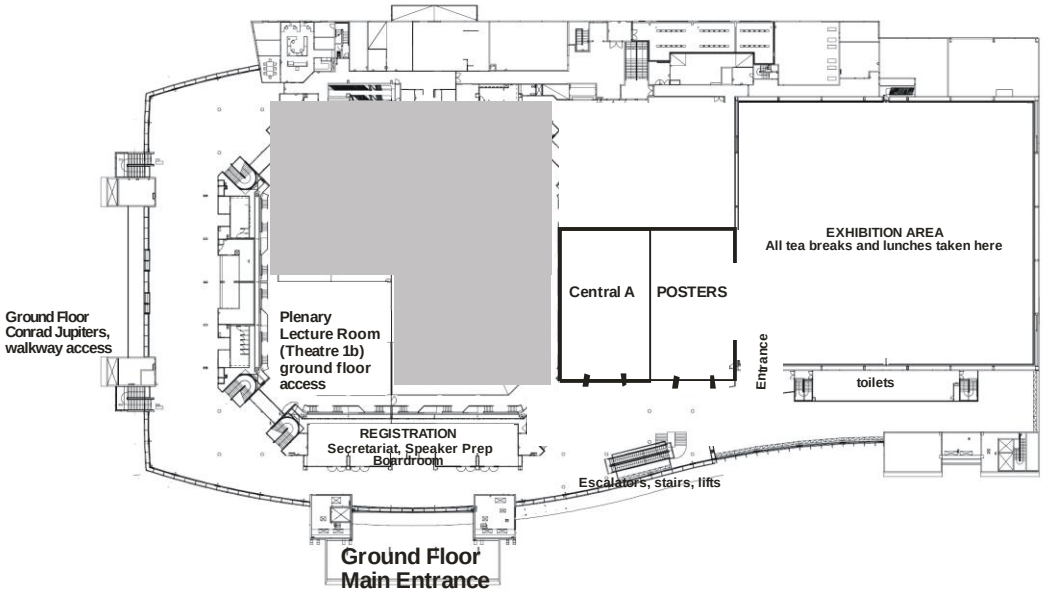
Gold Coast Convention and Exhibition Centre
Cnr Gold Coast Highway & TE Peters Drive
Broadbeach, Queensland 4218
Phone: +61 7 5504 4000
Fax: +61 7 5504 4001

The Gold Coast Convention and Exhibition Centre (GCCEC) is located in Broadbeach, on Australia's Gold Coast in Queensland. It is a unique destination which caters for both leisure and business tourism. Close to a variety of accommodation, shopping, restaurants, nightlife, entertainment and 70 km's of pristine white sandy beaches.

Session Locations

The Conference activities are spread out over two levels. Please refer to map below.





Organiser's Office and Registration Desk

The organiser's office and registration desk will be located on ground floor, main entrance of the Gold Coast Convention Centre. The office and desk will be attended at all times during the conference from 7:30am in the morning. Delegates should collect their satchel, name tag and other conference material on arrival. A message board will be placed immediately inside the main Exhibition entrance.

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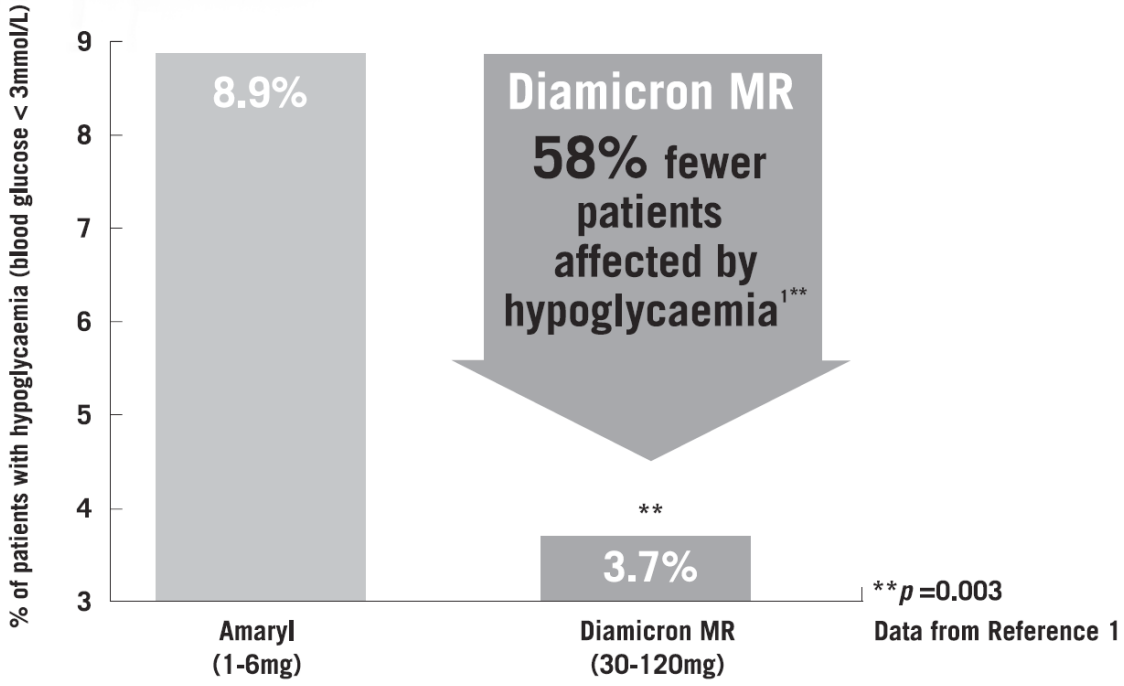

CONTROL WITH CONFIDENCE[1]

PBS Information: This product is listed on the PBS as an oral blood-glucose-lowering drug for Type 2 diabetes.

DIAMICRON MR® Abridged Product Information. Before prescribing, please refer to Approved Product Information. Indications: Type II diabetes, in association with dietary measures. **Contraindications:** Type I diabetes; diabetes keto-acidosis or coma, severe renal or hepatic impairment, pregnancy and lactation; concomitant miconazole, hypersensitivity to sulphonylureas or sulphonamides. **Precautions:** hypoglycaemia may occur with sulphonylureas, especially with alcohol intake or following strenuous exercise and be harder to recognise in the elderly or those receiving beta-blockers. Corrective therapy over several days may be necessary. Regular and adequate food intake is required. Fever, trauma, infection or surgery may jeopardise glycaemic control. Patients may require insulin and stopping Diamicron MR therapy may be necessary. Age, poor nutrition, adrenal and pituitary insufficiency increase sensitivity to anti-diabetic agents. **Use in Pregnancy:** Category C. Do not use in pregnancy. **Interactions:** drugs that affect blood sugar control such as thiazide diuretics, barbiturates, chlorpromazine, danazol, glucocorticoids, oestrogens and progestogens, salbutamol, terbutaline should be used with caution. Caution with alcohol and drugs that potentiate hypoglycaemia, eg, insulin, biguanides, sulphonamides, high dose salicylates, MAOI's, beta-blockers, cimetidine, clofibrate, ACE inhibitors, coumarin derivatives, chloramphenicol, ethanol, fluconazole and miconazole (see Contraindications). **Adverse Reactions:** mild and transient. Mild hypoglycaemia may occur but severe hypoglycaemia is uncommon. Occasional G.I. disturbances, skin reactions and elevations of serum creatinine, urea, bilirubin and hepatic enzymes have been reported. **Less common reactions:** consult Approved Product Information **Dosage and Administration:** for adults only. 30mg (1 tablet) – 120mg (4 tablets) daily with breakfast in a single dose. No dosage adjustment required in patients aged >65 years or with mild-moderate renal impairment. Tablets should not be broken or chewed. **Presentation:** white oblong modified release tablets containing gliclazide 30mg, in packs of 100. Store in a dry place below 30°C. **Date of Preparation:** 16th December 2002. **Sponsor:** Servier Laboratories (Australia) Pty. Ltd. 8 Cato Street Hawthorn, VIC 3122. Customer Service (Toll Free) 1800 33 1675. **PBS Dispensed Price – April 2006: $14.58, 100 + 5 repeats**

1. Schernthaner G, Grimaldi A, Di Mario U, et al. *Eur J Clin Invest*. 2004; 34: 535-524

DMMR1/0T TAC2234

The Speaker Preparation Room
The speaker preparation room is accessed through the organiser's office. Networked computers in this room will allow MS PowerPoint presentations loaded here to be shown in any of the session rooms. Technicians and assistants will be in attendance in the room and speakers are encouraged to load their presentations as soon as possible to avoid any last minute rushes.

Registration
Conference delegates receive the following services as part of their registration:

- Access to all scientific sessions on day(s) of registration
- A satchel with a copy of the delegate handbook and abstracts*
- Lunches on Monday, Tuesday and Wednesday
- Morning teas on Monday, Tuesday and Wednesday
- Afternoon teas on Monday and Tuesday
- The Welcome Function on Sunday evening

*All delegates receive a copy of the proceedings, but satchels can only be given to trade delegates if supply allows

Name Tags
Delegates are required to wear their name tags to all scientific and catered sessions.

Poster Viewing
Delegates with posters can find the correct position for their poster by finding the appropriate abstract number on the display panels in Central BC on the ground floor of the Gold Coast Convention Centre (adjacent to the exhibition area). 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 after the day's sessions on Tuesday. During formal poster discussions, the presenters should be present at their poster to answer questions and meet colleagues with similar research interests.

Social Functions

- The **Welcome Function** is in the Gold Coast Convention Centre on the Sunday evening from 6pm. Light refreshments and drinks will be served and the function is complimentary for all registration types.
- The **Women in Endocrinology Function** will follow the Welcome Function at 7pm. Again light refreshments and drinks will be served. This is a ticketed function and they must be purchased in advance.
- The Monday night **Student Function** is being held at Bojangles, Broadbeach (across the road from Gold Coast Convention Cente). Those who have already purchased a ticket should find their ticket with their registration papers on arrival. The ticket cost includes your meal, entertainment and drinks for the first three hours. The function begins at 7:30pm and dress is neat casual. This is a ticketed function and they must be purchased before the night.
- The **Conference Dinner** will be held onsite at the Gold Coast Convention Centre, first level, Foyer E. Pre-dinner drinks will be served from 7:00pm for a 7:30pm start. Dress is neat casual. Entertainment for the night is 'Don't Fret'. This is a ticketed function and they must be purchased in advance.

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.

Delegate Passport Competition - ASN is again sponsoring a delegate passport competition. Your entry form is in your satchel. Place completed entries in the competition box at the registration desk by 5pm Tuesday.

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.

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Sunday, 20 August 2006

SRB Workshop - Talking Science to the Media

2:00 PM - 3:30 PM M5 & M6
Chair: Sarah Meachem
Presenter: Sam Elan, Media Manouvres

Afternoon Tea

3:30 PM - 4:00 PM 1st Floor Foyer

SRB Symposium - Where Genotype Meets Phenotype; The New Frontier

4:00 PM - 6:00 PM M5 & M6

Co-Chairs: Grant Montgomery and Jean Fleming

4:00pm **John Mattick**
The role of non-coding RNA in human development *abs#001*

4:40pm **Peter Koopman**
Why men make sperm and women make oocytes: Discovery of the molecular signals controlling germ cell fate during embryonic development *abs#002*

5:10pm **Gail Risbridger**
A novel approach to study human prostate development and its diseases *abs#003*

ESA / SRB Welcome Function

6:00 PM - 7:30 PM 1st Floor Foyer

ESA / SRB Women in Endocrinology Function

7:00 PM - 8:00 PM M4

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first insulin[1] 1920's

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- Greater HbA_{1c} reduction*^
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*Humalog Mix 25 compared to glargine[4] ^ Humalog Mix 25 compared to NPH[5] † Humalog Mix 25 compared to Human Mix 30/70[6]

PBS Information: General Benefit. Refer to PBS Schedule for full PBS listing information for each product

Please review the Product Information before prescribing. Full PI available on request from trade display.

Humalog contains insulin lispro Rbe. Humalog Mix25 contains 25% insulin lispro and 75% insulin lispro protamine suspension Rbe. Humalog Mix50 contains 50% insulin lispro and 50% insulin lispro protamine suspension Rbe. PBS Dispensed Price: Humalog, Humalog Mix25 & Humalog Mix50 cartidges=$270.60, Humalog vials=$162.70.

INDICATIONS: Treatment of diabetes mellitus. **CONTRAINDICATIONS:** Hypoglycaemia, Hypersensitivity to insulin lispro or one of its excipients. Intravenous administration. **PRECAUTIONS:** Should not be mixed with other insulins. Any change of insulin or human insulin analogue should be made under medical supervision. **ADVERSE REACTIONS:** Hypoglycaemia, allergic reactions and lipodystrophy. **DOSAGE:** As determined by physician. Subcutaneous injection. Dose adjustments may be necessary in some circumstances – Refer to full PI. Should be taken before meals or soon after the meal. **References:** 1. White J, *et al.* Postgrad Med 2003;(6)113. 2. Puttaguntа AL *et al. Canadian Med Assoc J* 1998;158:506-511. 3. Approved Huma Product Information. 4. Malone JK, *et al. Diabetic Medicine* 2005;22:374-381. 5. *Malone JK, et al. J App Therapeutic Res* 2004;4(4):19-25. 6. Roach P. *et al. Clin Pharmacokinetics* 2002;41(13):1043-1057.

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Lilly

ESA Taft Plenary

8:30 AM - 9:30 AM Arena 1B

Chair: Jeffrey Zajac

Sadaf Farooqi

Lessons from Monogenic Obesity Syndromes *abs#004*

SRB Orals - Male Reproductive Tract

8:30 AM - 9:45 AM M5

Co-Chairs: Richard Ivell and Moira O'Bryan

8:30am **Pradeep Tanwar**
Bone morphogenetic protein-4 immunolocalization is developmentally regulated in mice testis *abs#201*

8:40am **Laura Parry**
Relaxin deficiency does not result in developmental abnormalities in the prostate gland of adult mice *abs#202*

8:50am **Marilyn Renfree**
The effects of oestrogen on mammalian sexual determination *abs#203*

9:00am **Hongshi Yu**
Characterization and Expression Patterns of *WNT4* During Gonadal Development in the Marsupial, *Macropus Eugenii* *abs#204*

9:10am **Jennifer Scott**
Presence of TGF-β but not IL-8 or GM-CSF in ram seminal plasma *abs#205*

9:20am **Nanette Schneider**
The sense of smell in the reproduction of the tammar wallaby (Macropus eugenii) *abs#206*

9:30am **Phillip Matson**
The effect of poly-l-lysine and urine volume upon the adhesion of numbat and dibbler sperm to microscope slides. *abs#207*

SRB Orals - Placental Development and Function

8:30 AM - 9:45 AM M6

Co-Chairs: Neil Gude and Amanda Sferruzzi-Perri

8:30am **Kirsty Pringle**
Oxygen, Insulin-Like Growth Factor-II (IGF-II) and their Interactions in Murine Trophoblasts *in vitro* *abs#208*

8:40am **Joanna James**
The effects of oxygen concentration and gestational age on extravillous trophoblast outgrowth from first trimester villous explants *abs#209*

8:50am **Eleanor Ager**
The Function and Evolutionary Significance of Genomic Imprinting in the Marsupial Placenta *abs#210*

9:00am **Neil Gude**
Proteomic analysis of human placenta identified increased expression of chloride intracellular channel 3 with pre-eclampsia *abs#211*

9:10am **Lloyd White**
Caspase-14: a new player in cytotrophoblast differentiation *abs#212*

9:20am **Kaori Koga**
Cyclic AMP stimulates soluble vascular endothelial growth factor receptor 1 (sVEGFR-1) production in human cytotrophoblast. *abs#213*

9:30am **Gayathri Rajaraman**
Opposing effects of HGF and TGF-β on HLX1 expression in human trophoblast cells *abs#214*

ESA Servier Award

9:30 AM - 9:45 AM — Arena 1B

Chair: Gail Risbridger

The 2006 Servier Award winner is Renea Taylor
Formation of Human Prostate Tissue from Embryonic Stem Cells

Morning Tea

9:45 AM - 10:15 AM — Exhibition Hall

ESA Novartis Junior Investigator Award Finalists

10:15 AM - 11:45 AM — Arena 1B

Co-Chairs: Jeffrey Zajac and Amy Au — **Session sponsored by Novartis**

10:15am **David MacIntyre**
Evidence for a novel mechanism for human myometrial activation *abs#101*

10:30am **Patrick Lim**
The classical androgen receptor pathway in Sertoli cells is vital for complete spermatogenesis *abs#102*

10:45am **Kirsten McTavish**
Evidence that premature infertility in transgenic FSH female mice is due to age-related changes in embryo survival: ageing oocyte or uterus? *abs#103*

11:00am **Christina Jang**
Reduced 11β Hydroxysteroid Dehydrogenase-1 in Skeletal Muscle in type 2 Diabetes: Induction by Dexamethasone *abs#104*

11:15am **Rachael O'Dowd**
Postnatal Growth is Improved by Cross-Fostering a Pup Born Small onto a Mother With Normal Lactation by Altering Alveolar Area, Milk Production and Milk Protein Gene Expression *abs#105*

11:30am **Sean Yang**
Regulation of voltage-gated Ca^{2+} currents of rat somatotropes through subtypes of somatostatin receptors *abs#106*

SRB Orals - Pregnancy and Fetus

10:15 AM - 12:00 PM — M5

Co-Chair: Larry Chamley and Guiying Nie

10:15am **Viv Perry**
The effect of maternal protein during pregnancy on birth weight in the bovine *abs#215*

10:25am **Brandon Menzies**
Distribution of ghrelin secreting cells in the stomach of the developing marsupial, *Macropus eugenii* *abs#217*

10:35am **Lenka Vodstrcil**
Restricting uterine blood flow in late pregnant rats results in an increase in uterine relaxin receptor (Lgr7) expression *abs#218*

10:45am **Sarah Robertson**
Systemic maternal awareness of conceptus antigens in pregnancy *abs#219*

10:55am **Katrina Hadfield**
Investigation of Maternal Immune Function Throughout Pregnancy *abs#220*

11:05am **Claire Roberts**
Maternal insulin-like growth factor-I and -II act via different pathways to increase fetal growth near term *abs#221*

11:15am **Amanda Sferruzzi-Perri**
Maternal IGF treatment in early to mid pregnancy has sustained effects on placental transport & nutrient partitioning near term *abs#222*

11:25am **Kathryn Hale**
HtrA3, a serine protease, in human pregnancy serum. *abs#223*

SRB Orals - Growth Factors and Signalling

10:15 AM - 12:00 PM M6

Co-Chair: Ravinder Anand-Ivell and Wendy Ingman

10:15am **Ravinder Anand-Ivell**
Characterizing the relaxin receptor (RXFP1) and the relaxin signaling pathway in human uterine cells. *abs#224*

10:25am **Stephen Anderson**
Increased expression of suppressors of cytokine signalling (SOCS) in the rat ovary during pregnancy *abs#225*

10:35am **Mai Sarraj**
Expression of Follistatin like -3 in Developing Mouse Gonads *abs#226*

10:45am **Laura Parry**
The lethal phenotype in relaxin-deficient (Rlx-/-) mice is due to abnormal nipple growth, and not impaired mammary gland structure or function. *abs#227*

10:55am **Wendy Ingman**
Null mutation in transforming growth factor beta1 impairs mammary gland development and impedes lactation *abs#228*

11:05am **Jinwei Chung**
Insulin family receptors in the developing marsupial *abs#229*

11:15am **Anette Szczepny**
Disruption of Hedgehog signalling in the adult mouse testis *abs#230*

11:25am **Mark Hedger**
Regulation of spermatogonial proliferation by interleukin-1 and activin a *in vitro*: a re-examination using an antagonist approach *abs#231*

SRB Founders Lecture

12:00 PM - 1:00 PM Arena 1B

Chair: Lois Salamonsen **Session sponsored by RFD**

Marilyn Renfree
Life in the pouch: Womb with a view *abs#005*

ESA Clinical - Meet the Expert 1: Vitamin D Across the Ages

12:00 PM - 1:00 PM M7

Chair: Roderick Clifton-Bligh **Session sponsored by GSK**

Terry Diamond
Vitamin D across the Ages *abs#006*

Lunch

1:00 PM - 2:00 PM Exhibition Hall

Session sponsored by Novo Nordisk

ESA Monday Poster Session - Pituitary, Reproduction, Pregnancy, Clinical I

2:00 PM - 3:00 PM Central BC

For poster listing, see end of Monday

SRB / ANZPRA Symposium - Growth Factor Regulation of Implantation and Placental Development

2:00 PM - 3:30 PM M5

Co-Chair: Claire Roberts and Padma Murthi **Session sponsored by Eli Lilly**

2:00pm **Claire Roberts**
The pivotal role of IGF-II in placental invasion, growth and function *abs#007*

2:30pm **Euan Wallace**
Macrophage inhibitory cytokine-1: roles in trophoblast function and decidual preparation *abs#008*

3:00pm **Evdokia Dimitriadis**
Interleukin 11 and Leukemia Inhibitory Factor: Mechanisms and Interactions in Implantation and Placentation *abs#009*

SRB Orals - Endocrine Regulation of Reproductive Function

2:00 PM - 3:30 PM M6

Co-Chair: Christopher Grupen and Chris Scott

2:00pm **Penelope Hawken**
Ram introduction stimulates pulsatile LH secretion in cyclic ewes *abs#232*

2:10pm **Eva Szarek**
Development of anterior pituitary cells and colocalisation of TSHβ and FSHβ with LHβ-immunoreactivity in the late gestational sheep fetus after disconnection of the hypothalamus and pituitary *abs#233*

2:20pm **Chris Scott**
Differentiating the sites of action of testicular steroids in the regulation of GnRH secretion and mating behaviour in rams: A model *abs#234*

2:30pm **Susan Jones**
Modulation of adrenal responsiveness in gestating females of *Egernia whitii*, a viviparous skink *abs#236*

2:40pm **Emily Hynes**
The contraceptive mechanism of levonorgestrel in a marsupial species *abs#237*

2:50pm **Christopher Grupen**
An effective superovulation protocol for the marmoset monkey (*Callithrix jacchus*) *abs#238*

3:00pm **David Armstrong**
Prostaglandin E_2 up-regulates luteinizing hormone receptor (LHR) expression and enhances steroidogenic responses of follicle cells *abs#239*

ESA Basic Symposium - Nuclear Receptor Regulation and Endocrine Disease

3:00 PM - 5:00 PM M7

Co-Chair: Anne Nelson and Charles Allan

3:00pm **Peter Fuller**
Determinants of Tissue and Ligand Selectivity in the Mineralocorticoid Receptor *abs#010*

3:30pm **Colin Clyne**
Roles of orphan receptor LRH-1 in reproduction and cancer *abs#011*

4:00pm **Edith Gardiner**
Vitamin D-Wnt pathway interactions in skeletal and non-skeletal cells *abs#012*

4:30pm **Gary Leong**
Nuclear Receptors in Metabolism: the Ski phenotypes and the NORphans *abs#013*

ESA Clinical Symposium - Pituitary disease: A Wide Spectrum of Disorders

3:00 PM - 5:00 PM Arena 1B

Co-Chair: Shaun McGrath and Roderick Clifton-Bligh **Session sponsored by Ipsen**

3:00pm **Warrick Inder**
Aspects in the Diagnosis and Management of Prolactinoma *abs#014*

3:30pm **Ken Ho**
Acromegaly: new frontiers in management *abs#015*

4:00pm **Steven Santoreneos**
Pituitary surgery with a pituitary tumour focus *abs#016*

4:30pm **Mark McLean**
Insights into Monitoring Therapy, and Challenges in Management of Hypopituitary Patients *abs#017*

ESA Orals - Diabetes, Obesity

3:00 PM - 5:00 PM M9

Co-Chair: David Kennaway and Brendan Waddell

3:00pm **Caitlin Wyrwoll**
A postnatal diet rich in omega-3 fatty acids attenuates glucocorticoid-programmed hyperinsulinemia but does not alter aberrant programmed gene expression in skeletal muscle. *abs#107*

3:15pm **David Kennaway**
A Lack of Peripheral Tissue Rhythmicity Alters Metabolic Homeostasis in Mice *abs#108*

3:30pm **Chen Chen**
Free fat acids (FFAs) stimulate Ca^{2+} release from IP_3-sensitive Ca^{2+} storage sites and reduce voltage-gated Ca^{2+} currents in primary cultured rat pancreatic β-cells *abs#109*

3:45pm **Helena Parkington**
Regional differences in endothelial dysfunction in diabetes mellitus *abs#110*

4:00pm **Nichola Thompson**
Preference between exercise and eating is influenced by prenatal nutrition and obesity development is prevented by providing an opportunity to exercise. *abs#111*

4:15pm **Jenny Chow**
The effect of estrogen on triglyceride homeostasis *abs#112*

4:30pm **Michele Gresham**
Circulating Porcine Ghrelin Concentrations are Responsive to Energy Metabolites and not Insulin *abs#113*

4:45pm **Elizabeth Doust**
Increased fat mass in androgen receptor knockout mice demonstrates a role for androgens in regulation of fat mass and metabolism in males *abs#114*

ESA / SRB Orals - Joint Female Reproduction

4:00 PM - 6:00 PM M5

Co-Chair: Simon Chu and Laura Parry

4:00pm **Ashwini Chand**
Inhibin α subunit with an A257T mutation is associated with premature ovarian failure: Is this inhibin form bioactive? *abs#115*

4:15pm **Tu'uhevaha Kaitu'u-Lino**
Neutrophil depletion retards endometrial repair *abs#116*

4:30pm **Kristy Brown**
Hydroxysteroid Sulfoconjugation as a Putative Determinant of Follicular Luteinization *abs#117*

4:45pm **Christine White**
Novel leukemia inhibitory factor antagonist blocks blastocyst implantation in the mouse *abs#118*

5:00pm **Ashwini Chand**
Laser Capture Microdissection and Array Analysis of Endometrium Identify CCL16 and CCL21 as Epithelial - Derived Inflammatory Mediators Associated with Endometriosis *abs#119*

5:15pm **Yin Lau Lee**
Hormonal Regulation and Convertase activities of complement 3 in human oviductal epithelial cells *abs#120*

5:30pm **Simon Chu**
The Role of Imatinib in the regulation of Granulosa Cell Tumour cell growth *abs#121*

5:45pm **Grant Montgomery**
Novel variants in human GDF9 in mothers of dizygotic twins *abs#122*

SRB Orals - Spermatogenesis and Sperm

4:00 PM - 6:00 PM M6

Co-Chair: Julia Young and Steve Johnston

4:00pm **Jennifer Ly**
Importin α2-recognised nuclear import in the control of spermatogenesis *abs#247*

4:10pm **Kim Lieu**
The importin-α2-dependent nuclear import mechanism of PSMC3IP and its possible role during spermatogenesis *abs#248*

4:20pm **Alison Graham**
Coexpression and potential interaction of the nuclear transporter importin β3 and nuclear pore complex component nucleoporin Nup153 in mouse testis. *abs#249*

4:30pm **Vinali Dias**
Differential expression of activin receptors in normal, hormone-treated, and neoplastic human testis. *abs#250*

4:40pm **Minjie Lin**
Ontogeny of cAMP-dependent tyrosine phosphorylation-signaling pathways during spermatogenesis and epididymal maturation in the mouse *abs#251*

4:50pm **Jeanette Olejnik**
PGP 9.5 as a marker for germ cell development in pre-pubertal and irradiated sheep testes. *abs#252*

5:00pm **Brian Setchell**
Long-term effects on the testis of a short period of unilateral cryptorchidism in rats *abs#253*

5:10pm **YengPeng Zee**
Assessment of Koala Sperm Mitochondrial Function with JC-1 *abs#254*

5:20pm **Steve Johnston**
One-sided ejaculation of sperm bundles in the echidna *abs#255*

5:30pm **Phillip Matson**
The objective assessment of porcine epididymal sperm using the sperm quality analyzer IIB. *abs#256*

SRB Post Doc Meeting
6:00 PM - 6:30 PM M5

ESA AGM
6:00 PM - 6:30 PM M7

SRB Student Meeting
6:00 PM - 6:30 PM M6

ESA / SRB Student Function
7:00 PM - 12:00 PM BOJANGLES, Broadbeach

FUTURE MEETINGS

2007

ESA Seminar 2007
4th - 6thMay 2007
Sebel Lodge Yarra Valley, VIC
www.esaseminar.org.au

ESA Clinical Weekend 2007
31st August – 2nd September 2007
New Zealand
www.esaclinicalweekend.org.au

Combined ESA/SRB Annual Scientific Meeting
2ND – 5TH September 2007
Christchurch Convention Centre, NZ
www.esa-srb.org.au

Monday posters

Pituitary

Carol Lee
Microarray analysis of growth hormone responsive genes in peripheral blood leukocytes in vivo *abs#401*

Simon Chu
Generation and validation of an FSH-β-promoter construct to target Cre Recombinase expression to anterior pituitary gonadotropes *abs#402*

Rachael Augustine
Pregnancy-induced leptin resistance involves a loss of appetite suppression by alpha-melanocyte stimulating hormone *abs#403*

Sally Newsome
Cushing's syndrome in a diabetic clinic population *abs#404*

David Grattan
Progesterone withdrawal triggers increased prolactin secretion and induction of SOCS mRNA in the arcuate nucleus during late pregnancy *abs#405*

Olivia Wynne
Impact of neonatal infection on CRH and glucocorticoid receptor mRNA abundance in the mouse brain. *abs#406*

Elizabeth Rivalland
Activation of corticotrophin-releasing hormone (CRH), arginine vasopressin (AVP) and enkephalin (ENK) neurones located in the paraventricular nucleus by isolation and restraint stress in sheep *abs#407*

Reproduction / Pregnancy

M Gould
The effects of a high phytoestrogen diet on the testes of the rat *abs#408*

Jorge Tolosa
Do Exosomes from Placental Explants Carry Syncytin? *abs#409*

Mark Green
Transgenerational effects of developmental programming on the fetal and placental characteristics of the rat. *abs#410*

Alexandra Umbers
Differential Expression of the Betaglycan Gene in the Murine Ovary and Testis during Gonadogenesis. *abs#411*

Naomi Scott
Differences in tumour necrosis factor alpha production by term and preterm placentae *abs#412*

Vicki Clifton
Placental gene expression is altered by fetal sex, maternal asthma and inhaled glucocorticoid intake *abs#413*

Keely McNamara
Lobe-specific reduction in mature prostate structural and functional differentiatiation in prostate epithelial specific androgen receptor knockout (PEARKO) mice *abs#414*

Jane Girling
Uterine expression of vascular endothelial growth factor-A mRNA in hormone treated ovariectomised mice *abs#415*

Isabelle Hoong
Developmental expression of peroxisome proliferator-activated receptors α and γ in the bovine placenta *abs#416*

Ning Li
Steroidogenic potential of the bovine fetal adrenal at early to mid-gestation *abs#417*

Michael Stark
The Impact of Antenatal Corticosteroids on Umbilical Venous and Arterial Cortisol in Neonates 23-41 Weeks Gestation *abs#418*

Clinical I

Tuck Yong
Dilated cardiomyopathy in a patient with Cushing's Syndrome *abs#419*

Namson Lau
Acromegaly and Thyroid Size *abs#420*

Ashish Gargya
The use of pre-operative ultrasound mapping of cervical lymph nodes to guide surgery for persistent and recurrent papillary thyroid carcinoma *abs#421*

Ashish Gargya
The Diagnostic Value of Neck Ultrasound and Thyroglobulin Measurement in the Fine Needle Aspirate from Cervical Lymph Nodes in the Follow-up of Patients with Papillary Thyroid Carcinoma *abs#422*

John Burgess
The Prevalence of Thyroid Disease and Related Risk Factors in Tasmania *abs#423*

Georgina Stilwell
The Influence of Gestation on Urinary Iodine Excretion in Pregnancy *abs#424*

John Wentworth
Implementation and evaluation of a protocol for reduced glucocorticoid replacement in pituitary surgery. *abs#425*

Jui Ho
The effect of weight loss on blood pressure, salt sensitivity and adrenocortical function *abs#426*

Walter Plehwe
Prevalence of Vitamin D Deficiency in Patients Undergoing Primary Elective Knee and Hip Arthroplasty. *abs#427*

actos™

pioglitazone hydrochloride

Once-daily ACTOS[1]:

✔ **Offers sustained HbA_{1C} reduction[2]**

- Equal to metformin at 1 year[3]
- Superior to gliclazide at 1 year and sustained superiority over 2 years[2]

✔ **Improves the diabetic lipid profile*[4]**

✔ **Directly targets insulin resistance[1]**

*Actos is not a lipid lowering agent. Practitioners should follow usual practice for treating dyslipidemia.

PBS Information: Authority required. Refer to PBS schedule for full information.

Before prescribing, review Full Product Information, available on request from the manufacturer.

INDICATIONS: Treatment of Type 2 diabetes mellitus inadequately controlled by diet as a single agent or in combination with sulfonylureas, metformin or insulin. **CONTRAINDICATIONS:** Patients with known hypersensitivity or allergy to ACTOS or any of its excipients. **PRECAUTIONS:** ACTOS should not be used in type 1 diabetes or for the treatment of diabetic ketoacidosis. To avoid hypoglycaemia, reducing the dose of concomitant agents may be necessary when ACTOS is combined with insulin or oral hypoglycaemic agents. In premenopausal anovulatory patients with insulin resistance, treatment with thiazolidinediones, including ACTOS, may result in resumption of ovulation. These patients may be at risk of pregnancy. ACTOS should be used with caution in patients with oedema. In placebo controlled clinical trials oedema was reported more frequently in patients treated with ACTOS than in placebo treated patients. ACTOS is not indicated in patients with NYHA Class III or IV cardiac status. ACTOS can cause fluid retention when used alone or in combination with other antidiabetic agents. Fluid retention may lead to or exacerbate heart failure. Patients should be observed for signs and symptoms of heart failure. ACTOS should be discontinued if any deterioration in cardiac status occurs. Therapy should not be initiated if patients exhibit clinical evidence of active liver disease or increased transaminase levels (ALT > 2.5 times the upper limit of normal) at the start of therapy. Dose related weight gain was seen with ACTOS alone and in combination with other hypoglycaemic agents. Use in Pregnancy – Pregnancy Category B3. ACTOS should be used during pregnancy only if the potential benefits justify the potential risk to the foetus. ACTOS should not be administered to lactating women. Safety and effectiveness in paediatric patients have not been established. **SIDE EFFECTS** (> 5% incidence): Upper respiratory tract infection 8.7%, headache 7.0%. Cardiac failure (very rare), hepatic failure (very rare). **DOSAGE AND ADMINISTRATION:** Once daily with or without food. Monotherapy: 15mg or 30mg od increasing after four weeks, if greater therapeutic effect is needed, to 45mg od. Combination: 30mg od in combination with sulfonylureas, insulin or metformin. If there is a particular risk of hypoglycaemia, ACTOS can be introduced at 15mg od. For patients on insulin, ACTOS should be introduced at 15mg od. Dosage can then be increased cautiously. Maximum **Recommended Dose:** 45mg od. Patients with Hepatic Impairment: start at 15mg od and increase cautiously. **PBS Dispensed Price:** 15mg $61.70, 30mg $92.34, 45mg $118.64

References: 1. ACTOS Approved Product Information. 2. Tan MH et al. Comparison of pioglitazone and gliclazide in sustaining glycemic control over 2 years in patients with type 2 diabetes. Diabetes Care, 2005; 28:544-550. 3. Schernthaner D et al. Efficacy and safety of pioglitazone versus metformin in patients with type 2 diabetes mellitus: A double-blind, randomized trial. The Journal of Clinical Endocrinology and Metabolism 89(12):6067-6068. 4. Goldberg RB et al. A comparision of lipid and glycemic effects of pioglitazone and rosiglitazone in patients with type 2 diabetes and dyslipidemia. Diabetes Care;2005:28:1547-1554.

Lilly

SRB MLA Plenary Lecture

8:30 AM - 9:30 AM — Arena 1B

Chair: Graeme Martin — **Session sponsored by Meat Livestock Australia**

William Thatcher

Nutraceutical and pharmaceutical effects on uterine and hormonal responses associated with early pregnancy in lactating dairy cattle *abs#018*

ESA Clinical - Meet the Expert 2: Iodine Sufficiency Across Australia: Do we currently make the grade?

8:30 AM - 9:30 AM — M5

Chair: John Walsh

Creswell Eastman

Iodine sufficiency across Australia: do we currently make the grade? *abs#019*

ESA Orals - Pregnancy, Parturition

8:30 AM - 9:30 AM — Central A

Co-Chair: Vicki Clifton and Julie Owens

8:30am **Elisa Tyson**
A Novel Mode of Action for Oxytocin and cAMP in Regulating Myometrial Contractility *abs#123*

8:45am **Kerryn Westcott**
Growth Restricted Fetal and Newborn Rats Have Altered Brain Neurosteroids *abs#124*

9:00am **Mary Wlodek**
Puberty Onset is Delayed Following Placental and Lactational Restriction *abs#125*

9:15am **Julie Owens**
Restriction of placental growth and size at birth increases pancreatic expression of the β-cell survival factor IGF-II. *abs#126*

Morning Tea

9:30 AM - 10:00 AM — Exhibition Hall

ESA / SRB Orals - Joint Male Reproduction Session

10:00 AM - 12:00 PM — M5

Co-Chairs: Kate Loveland and Mark Hedger

10:00am **Ulla Simanainen**
Prostate atrophy and abnormal epithelial cell proliferation due to targeted disruption of the prostate epithelial androgen receptor in PEARKO mice *abs#135*

10:15am **Geoffrey Shaw**
Androstanediol and development of the Wolffian ducts in tammars *abs#136*

10:30am **Yao Wang**
Anti-Activin Consequences of Glucocorticoid Action Within the Male Reproductive Axis *abs#137*

10:45am **Gurpreet Kaur**
Calmodulin-dependent nuclear import of the testis-determining factor SRY *abs#138*

11:00am **Jayne Sierens**
Liver Receptor Homologue-1 (LRH-1) regulated genes within the testis. *abs#139*

11:15am **Elspeth Gold**
Activin βC-subunit is a regulator of testis and liver function: implications for activin biology *abs#140*

11:30am **Yogeshwar Makanji**
Glycosylated Forms of Human Inhibin A and B Show Marked Differences in In Vitro Bioactivity *abs#141*

11:45am **Gerard Tarulli**
In vivo regulation of tight junction proteins by gonadotrophins in the adult Djungarian hamster testis *abs#142*

ESA Orals - Transcription, Signalling

10:00 AM - 12:00 PM Central A

Co-Chairs: Esme Hatchell and Gary Leong

10:00am **Stephen Myers**
Crosstalk Between the Chicken Ovalbumin Upstream Promoter Transcription Factors (COUP-TFs) and LXR In Skeletal Muscle Cells Regulates Lipid Homeostasis *abs#143*

10:15am **Carolyn Mitchell**
Chromatin Structure and NFκB Binding in the Prostaglandin Endoperoxide H Synthase (PGHS-2) Promoter in Term Fetel Membranes *abs#144*

10:30am **Brendan Waddell**
Interactive effects of fetal programming and postnatal dietary omega-3 fatty acids on methylation status of renal glucocorticoid receptor and 11β-hydroxysteroid dehydrogenase 2. *abs#145*

10:45am **Esme Hatchell**
Complex Interations Between SLIRP, A SRA-Binding Nuclear Receptor Corepressor, and other Nuclear Receptor Coregulators *abs#146*

11:00am **Richard Nicholson**
cAMP Regulates CRH Gene Expression Through a Multi-Element Response Unit *abs#147*

11:15am **Carmel Cluning**
The Prolyl Isomerase FKBP52 Enhances Glucocorticoid Receptor Signalling by Targeting a Conserved N-Cap Proline Critical for Helix 12 Dynamics *abs#148*

11:30am **Michael Pearen**
Evidence for crosstalk between the orphan nuclear receptor, NOR-1 and β-adrenergic signalling in skeletal muscle cells *abs#149*

11:45am **Jasmin Dromey**
Arrestin sensitivity of Class A and B G-protein coupled receptors (GPCRs) can be defined using extended BRET (eBRET) in different cell types *abs#150*

ESA Orals and Clinical Session: ESA Mayne Pharma Bryan Hudson Clinical Award

- the first two session presenters are Award Finalists

10:00 AM - 12:00 PM M8

Co-Chairs: Carolyn Allen and Mark McLean

10:00am **Peter Liu**
The rate, extent and modifiers of spermatogenic recovery after male hormonal contraception: an integrated analysis *abs#151*

10:15am **Jui Ho**
Relative Maternal Hypocortisolism in High Risk Human Pregnancy *abs#152*

10:30am **Roger Smith**
Rate of Change of Corticotrophin Releasing Hormone and hCG Nadir Provide Accurate Identification of Women At Risk of Preterm Birth *abs#153*

10:45am **Florence Law**
Proton Pump Inhibitors Cause Hypomagnesaemic Hypoparathyroidism *abs#154*

11:00am **John Walsh**
Small changes in thyroxine dosage do not produce measurable changes in hypothyroid symptoms, well-being or quality of life: results of a double blind, randomized clinical trial. *abs#155*

11:15am **Peter Ebeling**
Pamidronate or zoledronic acid reduce bone loss after allogeneic stem cell transplantation *abs#156*

11:30am **Jun Yang**
Multifocal Papillary Thyroid Carcinoma arising in Hashimoto's Thyroiditis *abs#157*

11:45am **Paul Lee**
Hyperserotoninaemia in Gilbert's Syndrome mimicking Carcinoid Syndrome -- A Novel Mechanism? *abs#158*

ESA Orals - HPA, Pituitary

10:00 AM - 12:00 PM M2

Co-Chairs: Catherine Coulter and Duncan Topliss

10:00am **Timothy Cole**
Direct effects of aldosterone *in vivo* on endothelin-1 gene expression in the kidney and colon *abs#127*

10:15am **Catherine Coulter**
Maternal Dexamethasone Treatment in Early Gestation Suppresses Steroidogenic Capacity and Growth of the Fetel Adrenal *abs#128*

10:30am **Matthew Doogue**
Salivary cortisol to monitor hydrocortisone treatment in patients in patients with hypoadrenalism *abs#129*

10:45am **Peter Mark**
Elevated P-glycoprotein expression limits glucocorticoid receptor response to cortisol and impedes dexamethasone transport across monolayers in placental BeWo cells *abs#130*

11:00am **Craig Harrison**
Activin-A Binds Follistatin and Type II Receptors Through Overlapping Binding Sites: Generation of Mutants with Isolated Binding Activities *abs#131*

11:15am **John Fitter**
Different Environmental Stresses Elicit Differential CRH Responses In Limnodynastes peronii *abs#132*

11:30am **Shoshana Sztal-Mazer**
Functional FSH-secreting adenoma in MEN1 *abs#133*

11:45am **Rachel Hill**
Lack of estrogen leads to a significant reduction in area and cell number of a region corresponding to the Sexually Dimorphic Nucleus (SDN) of the medial preoptic area in male and female mice of the Sv129J strain *abs#134*

SRB Symposium - Stem Cells in Reproductive Tissues

10:00 AM - 12:00 PM Arena 1B

Chair: Caroline Gargett and Renea Taylor **Session sponsored by Australian Stem Cell Centre**

10:00am **Melissa Little**
Searching for stem cells in the kidney *abs#020*

10:30am **Jane Visvader**
Identification of mammary stem cells and their role in breast cancer. *abs#021*

11:00am **Jock Findlay**
Germline stem cells in the ovary *abs#022*

11:30am **Caroline Gargett**
Adult Stem/Progenitor Cells in the Endometrium *abs#023*

ESA Harrison Lecturer

12:00 PM - 1:00 PM Central A

Chair: Jeffery Zajac

William Crowley
New Genes that Control Reproduction in the Human and Their Genotype-Phenotypes: Evidence from Human Disease Models *abs#024*

SRB lunch including Student Lunch - with the Founder's Lecturer

12:00 PM - 1:00 PM M5

Collect Lunch first from the Exhibition Hall

SRB Orals - Oocytes and Ovarian Function

1:00 PM - 3:00 PM Central A

Co-Chairs: Ray Rodgers and Ann Drummond

1:00pm **Raymond Rodgers**
Gene expression profiling by microarray analyses of bovine granulosa cells from small and large healthy antral follicles *abs#265*

1:10pm **Helen Irving-Rodgers**
Correlative gene expression and follicular dominance in the bovine *abs#266*

1:20pm **Ann Drummond**
Mechanism by whicH FGF9 stimulates ovarian progesterone production *abs#267*

1:30pm **Chris Edgecumbe**
The localisation of cyclin B1 and fat facets in mouse (FAM) in murine oocytes undergoing maturation in vitro *abs#268*

1:40pm **Cheryl Schelbach**
Improved development of murine embryos derived from COCs matured with the O-linked glycosylation inhibitor, BADGP. *abs#269*

1:50pm **Kelly Banwell**
Fetal and placental outcomes are programmed by oxygen concentration during maturation of murine oocytes. *abs#270*

2:00pm **Michael Boden**
Changes in ovarian gene expression and mammary development in the *BMAL1* knockout mouse *abs#271*

2:10pm **Jean Fleming**
Kallikrein 4 expression in mouse ovaries with serous inclusion cysts. *abs#272*

2:20pm **Marissa Bowden**
Expression of Serine Protease Htra3 During Ovarian Development and Folliculogenisis in the Rat *abs#273*

2:30pm **Carolina Viñoles**
Use of a Single-Follicle-Wave Cycle to Study Acute Effects of Changes in Nutrition on Ovulation Rate in Ewes *abs#274*

SRB Orals - Uterus and implantation

1:00 PM - 3:00 PM M5

Co-Chairs: Eva Dimitriadis and John Bromfield

1:00pm **Alejandro Tapia**
Identification of genes in human endometrial and stromal cells that alter during the aquisition of receptivity *abs#275*

1:10pm **Chelsea Stoikos**
Activin inhibitors retard human endometrial stromal cell decidualisation *abs#276*

1:20pm **Laura Lindsay**
Fluid transport in the rat uterus during early pregnancy *abs#277*

1:30pm **Joanna Biazik**
Evolution of viviparity in reptiles: role of tight junctions of the uterine epithelium *abs#278*

1:40pm **John Bromfield**
Seminal plasma regulates MMP-2, MMP-3 and VEGF-C mRNA expression in the peri-implantation mouse uterus *abs#279*

1:50pm **Shalini Panwar**
Leptin is critical for successful implantation in mice *abs#280*

2:00pm **Claudia Freyer**
Pro-protein convertases: novel regulators of endometrial physiology and implantation *abs#281*

2:10pm **Lynette Kilpatrick**
Identification of PC6 Substrates Involved in Human Stromal Cell Decidualisation Using Proteomics *abs#282*

2:20pm **Anita Ledgard**
Effect of polyunsaturated fatty acids on PGF 2alpha and PGE 2 synthysis in bovine endometruim and trophoblast tissues. *abs#283*

2:30pm **Claire Walker**
The Distribution of Opioid Receptors in the Human Placenta and Decidua from Early

Pregnancy *abs#284*

ESA Lunch

1:00 PM - 2:00 PM Exhibition Hall

Session sponsored by Eli Lilly

ESA Tuesday Poster Session - Androgens, Cancer, Metabolism, Adipocytokines, Clinical II Developmental, Immunology

2:00 PM - 3:00 PM Central BC

For poster listing, see end of Tuesday

Combined Neuroendocrinology Australasia /ESA Symposium: Kisspeptins in Neuroendocrinology

3:00 PM - 5:00 PM Arena 1B

Chair: Brian Oldfield **Session sponsored by DSL Australia / Beckman Coulter Australia**

3:00pm **Allan Herbison**
Kisspeptin activation of GnRH neurons to initiate puberty and ovulation. *abs#025*

3:30pm **William Crowley**
The Biology of Kisspeptins and GPR54 *abs#026*

4:00pm **Manuel Tena-Sempere**
Kisspeptin and GPR54: molecular conduits for puberty onset and central integration of energy balance and reproduction *abs#027*

4:30pm **Jeremy Smith**
The role of kisspeptin in mediating sex steroid feedback control of GnRH *abs#028*

ESA Orals - Androgens, Cancer

3:00 PM - 5:00 PM M8

Co-Chairs: Helen MacLean and Judith Clements

3:00pm **Lisa Butler**
Suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, represses androgen receptor expression and acts synergistically with an androgen receptor antagonist to inhibit prostate cancer cell proliferation *abs#159*

3:15pm **Tina Bianco-Miotto**
Expression of the Androgen Receptor and its Association with Disease Outcome in Breast Cancer *abs#160*

3:30pm **Sharyn Kelleher**
Older Men with Organic Androgen Deficiency (AD) Maintain Similar Trough and Peak Blood Testosterone Levels and Quality of Life as Younger AD Men without Change in Testosterone Implant Dose. *abs#161*

3:45pm **Angela Chew**
Mechanisms underlying inhibition of androgen-insensitive prostate cancer cell proliferation by peroxisome proliferator-activated receptor gamma. *abs#162*

4:00pm **Maggie Centenera**
Development of dominant negative androgen receptors as a novel therapeutic strategy for prostate cancer *abs#163*

4:15pm **Helen MacLean**
Global and muscle-specific androgen receptor knockout mice demonstrate direct anabolic actions of androgens in skeletal muscle *abs#164*

4:30pm **Mathis Grossmann**
Low Testosterone Levels are not Common in Men With Type 2 Diabetes Mellitus and, in Contrast to Low SHBG Levels, not Associated with Poor Glycaemic Control. *abs#165*

AACB / ESA Clinical Symposium - Laboratory Measures in Clinical Endocrinology Practice

3:00 PM - 5:00 PM M5

Co-Chairs: Gregory Ward and Shaun McGrath **Session sponsored by Servier**

3:00pm **Paul Glendenning**
Laboratory measures in clinical endocrine practice: Bone markers *abs#029*

3:30pm **David Handelsman**
Testosterone measures - integration of clinical and analytical issues *abs#030*

4:00pm **Paul Williams**
Macroprolactin *abs#031*

4:30pm **Jim Stockijt**
Thyroid antibodies in clinical endocrinology *abs#032*

SRB Young Investigators

3:30 PM - 5:00 PM Central A

Chair: Lois Salamonsen **Session sponsored by Serono**

3:30pm **Rachel Chan**
Estrogen Stimulates Mouse Endometrial Stem-like Cell Proliferation *abs#285*

3:40pm **Natalie Hannan**
A novel role for fractalkine in regulating human trophoblast extracellular matrix and adhesion molecules. *abs#286*

3:50pm **Premila Paiva**
Interleukin-11 promotes migration but not proliferation of human trophoblast cells *abs#287*

4:00pm **Saleela Ruwanpura**
Accelerated apoptosis is the cause of germ cell loss in gonadotrophin-suppressed men *abs#288*

4:10pm **Kirsty Walters**
Androgen receptor is not essential for female reproduction but plays important roles in optimising follicle development and ovulation *abs#289*

4:20pm **Camryn Allen**
Successful artificial insemination (AI) in the koala using neat and extended semen collected by electroejaculation (EE) *abs#290*

ESA Lecture

5:00 PM - 6:00 PM Arena 1B

Chair: Leon Bach

Peter Leedman
Nuclear receptor coregulators – getting to the heart of hormone action *abs#033*

ESA \ SRB Conference Dinner - Gold Coast Convention Centre

7:30 PM - 12:00 PM Foyer E upstairs

Tuesday Posters

Androgens

Julie McManus
Androgen mediated erythropoiesis occurs via classical androgen receptor signalling in male mice *abs#428*

Jayne Sierens
Oestrogen regulation of the Liver Receptor Homologue (LRH-1) within testicular cells. *abs#429*

Metabolism / Adipocytokines

Suryaprakash Raichur
'Gain of function' analysis in skeletal muscle cells suggests Retinoic Acid Receptor related Orphan Receptor Gamma controls metabolic gene expression. *abs#430*

Susan Millard
Regulation of Body Composition Development by the Ski Proto-Oncogene *In vivo* *abs#431*

Shu-Ching Wang
NR3B3 (ERRγ) controls pathways that regulate muscle mass and adiposity in skeletal muscle cells. *abs#432*

Melanie Tran
Increased intramyocellular lipid causes skeletal muscle insulin resistance in the young adult guinea pig of low birthweight *abs#433*

Yue Chen
Androgen regulation of satellite cell function in vitro *abs#434*

Clinical II

Michelle Gordon
Metastatic macroprolactinoma in multiple endocrine neoplasia type 1 (MEN-1) mimicking meningioma in cervical cord *abs#435*

Namson Lau
Ablative Radioiodine Therapy in a case of concurrent Thyroid Carcinoma, Graves' Disease with associated Ophthalmopathy and Juvenile Onset Glaucoma *abs#436*

Livia Rivera-Woll
Pituicytoma: An Unusual Primary Tumor of the Pituitary with Characteristic MRI and Histopathological Findings *abs#437*

Abdullah Omari
Metabolic Rehabilitation: A new approach to managing obese patients with Type 2 Diabetes Mellitus *abs#438*

Divina Brillante
Evidence for Functional Expression of Vascular AT2 Receptors in Patients with Insulin Resistance *abs#439*

David Hoffman
Electronic Audit in Private Endocrine Practice of Efficacy & Tolerability of Rosiglitazone in Triple Oral Therapy *abs#440*

Simon Rajaratnam
Clinical profile of patients with microalbuminuria *abs#441*

Ashley Makepeace
Screening for Phaeochromocytoma in Renal Failure *abs#442*

Constance Yap
Graves' disease in pregnancy: when more than joy alone can set your heart racing *abs#443*

Paul Lee
Multiple Insufficiency Fractures of the Pelvis and Femora in a Post-Menopausal Woman on Alendronate Therapy *abs#444*

Cancer

John Lai
The prostate-specific antigen (*PSA*) polymorphism (G-158A) alters interaction of androgen response element 1 with the androgen receptor *abs#445*

Brett Hollier
IGF-I:IGFBP:VN complex enhanced breast cancer cell migration involves both VN-binding integrins and the IGF-1R through activation of the AKT/PI3-K signalling pathway. *abs#446*

Rachael Murray
Expression of components of the ghrelin/growth hormone secretagogue receptor axis in breast cancer cell lines and in breast cancer histopathological specimens *abs#447*

Sheena Wong
Neuroblastoma Cell Differentiation by Fibroblast Growth Factor-2 (FGF-2) Involves Regulation Of Inhibitor Of Differentiation (ID) Genes And Suppressor Of Cytokine Signaling-2 (SOCS-2) *abs#448*

Vincenzo Russo
Neuroblastoma Cell Adaptation To Hypoxia Is Achieved Via Complex Modulation of Genes Enhancing Cell Survival and Metastasis. *abs#449*

Albert Frauman
CD151 Gene Knock-down Suppresses the Motility of Prostate Cancer Cell Line PC3: A Link to Prostate Cancer Metastasis? *abs#450*

Nicole Luk
Evidence for cross talk between melanocortin-1 receptor and NR4A nuclear receptor signalling in melanocytic cells. *abs#451*

Kenneth Ho
Malignant Insulinomas with Hepatic Metastases Successfully Treated with Selective Internal Radiation Therapy (SIRT). *abs#452*

Bradley Newell
CD151 – A novel clinical prognostic tumour marker in prostate cancer. *abs#453*

Developmental / Immunology

Nicholas Kasmeridis
Melatonin as an Immunoenhancing Vaccine Adjuvant *abs#454*

Michelle Lui
Is Defective Immunity a Feature of Alstrom Syndrome? *abs#455*

Pituitary

Jennifer Hansen
The Relationship between Body Composition and Physical Performance: a Study in Young Recreational Athletes. *abs#456*

Reproduction

Anita Ledgard
Legumain in early bovine placentation *abs#457*

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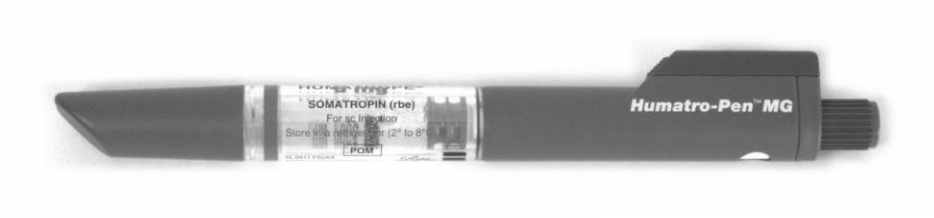

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Humatrope (Somatropin)

Indications Children who have growth failure, Turner syndrome, chronic renal insufficiency; Adults with severe growth hormone deficiency. **Dosage** To be administered subcutaneously. Intramuscular injection acceptable. Children- 0.177-0.255 mg/kg of bodyweight per week. Girls with Turner Syndrome- 0.3 mg/kg of body weight per week. Adults- 0.04mg/kg per week to a maximum of 0.08 mg/kg. **Contraindications** Evidence of activity of a tumor; allergy to meta-cresol or glycerol; growth promotion in children with closed epiphyses; patients with acute critical illness due to complications following open heart surgery or abdominal surgery, multiple accident trauma, or to patients having acute respiratory failure. **Precautions** Patients with growth hormone deficiency secondary to an intracranial lesion should be examined frequently for progression or recurrence of the underlying disease process. Patients should be observed for evidence of glucose intolerance. Children with diabetes should be carefully monitored during treatment. Patients with coexisting ACTH deficiency should have their glucocorticoid replacement dose carefully adjusted to avoid an inhibitory effect on growth. Patients should have periodic thyroid function tests and be treated with thyroid hormone when indicated. If papilloedema is confirmed, a diagnosis of benign intracranial hypertension should be considered and, if appropriate, growth hormone treatment should be discontinued. Any child with the onset of a limp during growth hormone therapy should be evaluated. The injection sites should be rotated to minimise the risk of lipoatrophy. Girls with Turner syndrome should be evaluated for otitis media and other ear disorders. **–Adverse Reactions** Paediatric Patients - oedema; injection site pain, lipoatrophy, hyperglycaemia; leukaemia; benign intracranial hypertension; localised muscle pain Turner Syndrome- hypothyroidism; peripheral oedema. Adult Patients- oedema/peripheral oedema; muscle pain; joint pain; paraesthesias, carpal tunnel syndrome; hyperglycaemia; headache; weakness; glycosuria.

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ESA & ADS Plenary

8:30 AM - 9:30 AM — Arena 1B

Chair: Terri Allen and Stephen Twigg

Eiichi Araki
Intracellular stress, mitochondrial dysfunction and diabetes complications *abs#034*

SRB Orals - Infertility and Pregnancy Pathologies

8:30 AM - 9:30 AM — M7

Co-Chairs: Jane Girling and Louise Hull

8:30am **Naomi Morison**
Mifepristone enhances endometrial repair in a mouse model for break-through bleeding associated with implanon use. *abs#291*

8:40am **Kylie Van der Hoek**
Osteoblast specific factor -2 and osteopontin are present in endometriotic tissues. *abs#292*

8:50am **Kathryn Visser**
Endometrial interleukin 11 is dysregulated in infertility during the implantation window. *abs#293*

9:00am **Rachael Nowak**
Interleukin-1β -511 polymorphism is associated with preeclampsia *abs#294*

9:10am **Katy Freed**
The gene encoding the constant region of the heavy chain of immunoglobulin G is differentially expressed in human decidua in association with preeclampsia *abs#295*

9:20am **Larry Chamley**
The role of the novel sperm protein SPRASA in infertility *abs#296*

SRB Orals - Germ Cells and Stem Cells

8:30 AM - 9:30 AM — M9

Co-Chairs: Peter Koopman and Rachael Chan

8:30am **Julia Young**
The influence of regulated nuclear transport on primordial germ cell lineage specification *abs#297*

8:40am **Danielle Hickford**
Primordial germ cell specification: an alternative mammalian model. *abs#298*

8:50am **Zhen Zhang**
Establishment of Molecular Markers for Bovine Testis Transplantation *abs#299*

9:00am **Muren Herrid**
Magnetic Activated Cell Sorting for Purification of Bovine Type A Spermatogonia *abs#300*

9:10am **Sirisha Mendis**
Activin effects germ cell number at specific developmental ages in the fetal mouse testis *abs#301*

9:20am **Sridurga Mithra-Prabhu**
Regulation of c-Kit receptor in germ cells of the rodent testis by members of TGF-beta superfamily *abs#302*

Morning Tea

9:30 AM - 10:00 AM — Exhibition Hall

ESA / ADS Symposium - Routes of Hormonal/Insulin Delivery

10:00 AM - 12:00 PM — Arena 1B

Co-Chairs: Paul Williams and Richard McIsaac — **Session sponsored by Schering**

10:00am **Eiichi Araki**
Novel approaches for the treatment of Diabetes -Wearable Artificial Endocrine Pancreas (AEP) and Mild Electric and Thermo Generator (MET)- *abs#035*

10:30am **Dennis Yue**
Inhaled Insulin in Diabetes Treatment *abs#036*

11:00am **Ann Simpson**
Insulin delivery by genetic engineering *abs#037*

11:30am **Ann Conway**
Testosterone delivery - routes of administration *abs#038*

ESA / SRB Symposium - Genetic Causes of Reproductive Failure

10:00 AM - 12:00 PM M6

Co-Chairs: Guiying Nie and Robert Norman

10:00am **William Crowley**
Hypothalamic causes of reproductive behaviour *abs#039*

10:30am **Kate Steinbeck**
Turner Syndrome *abs#040*

11:00am **Moira O'Bryan**
Genetic Causes of Reproductive Failure in the Male *abs#041*

11:30am **Guiying Nie**
Endometrial Proprotein Convertase 6: a Critical Regulator for Embryo Implantation *abs#042*

ESA Orals - Growth Hormone, IGFs, IGFBPs

10:00 AM - 12:00 PM M7

Co-Chairs: Vince Russo and Kin Leung

10:00am **Rebecca Pelekanos**
The Growth Hormone Receptor is Constitutively Dimerized and Activated by Rotation of the Cytoplasmic Domains *abs#166*

10:15am **Linda Kerr**
Removal of Box1 from the GH Receptor abolishes Jak/STAT signalling but not MAPK *abs#167*

10:30am **Kin-Chuen Leung**
Contrasting Regulatory Effects of Selective Oestrogen Receptor Modulators on GH Signalling in Breast and Kidney Tissues *abs#168*

10:45am **Kathryn Gatford**
Reduced IGFBP-3 abundance contributes to catch-up growth in the intrauterine growth-restricted lamb. *abs#169*

11:00am **Vincenzo Russo**
Insulin-Like Growth Factor Binding Protein-2 is an Essential Regulator of Neuroblastoma Cell Motility *abs#170*

11:15am **Kin-Chuen Leung**
GH Regulation of Metabolic Genes in Muscle: A Microarray Study in Hypopituitary Men *abs#171*

11:30am **Anne Nelson**
The influence of demographic factors on the ratio of 20-kDa and 22-kDa GH isoforms and the utility of the ratio for detection of GH doping in sport. *abs#172*

11:45am **Vita Birzniece**
Modulatory Effect of Raloxifene and Oestrogen on the Metabolic Action of GH in Hypopituitary Women *abs#173*

SRB Orals - Embryo Development

10:00 AM - 12:00 AM M9

Co-Chairs: Marie Pantaleon and Michelle Lane

10:00am **Christine Yeo**
Exogenous Growth Differentiation Factor 9 During In Vitro Maturation of Oocytes Improves Subsequent Embryonic Development and Fetal Outcome *abs#303*

10:10am **W.N. Chow**
Fertility study of Complement-3 In Mice *abs#304*

10:20am **Chris O'Neill**
Variable expressivity of the tumour suppressor protein P53 in human embryos *abs#305*

10:30am **Hugh Morgan**
Demonstration of an association between the extent of culture stress and the expression of P53 in mouse embryos. *abs#306*

10:40am **Vikram Tallapragada**
P53 expression in mouse and human sperm *abs#307*

10:50am **D. Rieger**
Comparison of a single medium with sequential media for the culture of sibling human embryos to the blastocyst stage *abs#308*

11:00am **Celine Lawler**
Significance of early developmental markers in human cryopreserved embryos *abs#309*

11:10am **Erica Little**
Characterization of E74 like factor 3 in the murine blastocyst *abs#310*

11:20am **Melanie Gibson**
The investigation of mRNA expression of nuclear importins during bovine preimplantation embryogenesis. *abs#311*

Lunch
12:00 PM - 1:00 PM Exhibition Hall

SRB / DE Symposium - Epigenetic Mechanisms in Programming Pre-implantation Embryos
1:00 PM - 2:30 PM M7 & M8

Co-Chairs: Chris O'Neill and Sarah Robertson

Session sponsored by ARC/NHMRC Network in Genes, Environment and Development

1:00pm **Lorraine Young**
Environmental Influences on DNA Methylation in Embryonic Cells: Investigating Mechanisms and Phenotypic Consequences. *abs#043*

1:30pm **Josie McConnell**
A mitochondrial component to developmental programming *abs#044*

2:00pm **Hugh Morgan**
Methylcytosine deamination by DNA deaminases and expression in reprogramming tissues. *abs#045*

Introduction to Thyroid Ultrasound Workshop
1:00 PM - 5:00 PM M9

Session sponsored by Genzyme, Sound Equipment, GE Healthcare, Siemens Medical and Toshiba

Registrations required

The Australian Pituitary Foundation, the Endocrine Society of Australia and an educational grant from Novartis, are conducting an acromegaly awareness campaign in an effort to facilitate easier recognition and diagnosis of this disease.
If you would like to receive copies of the campaign poster (below) or additional information please contact Novartis on acromegaly.awareness@novartis.com

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Endocrine Society of Australia (ESA) **Site 22**
SYDNEY NSW 2000
Phone: 02 9256 5405
www.racp.edu.au/esa
The Endocrine Society of Australia 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 8 members of its Council who are elected every two years by a ballot of the membership in accordance with the Constitution. Visit the site to meet the Endocrine Society secretariat.

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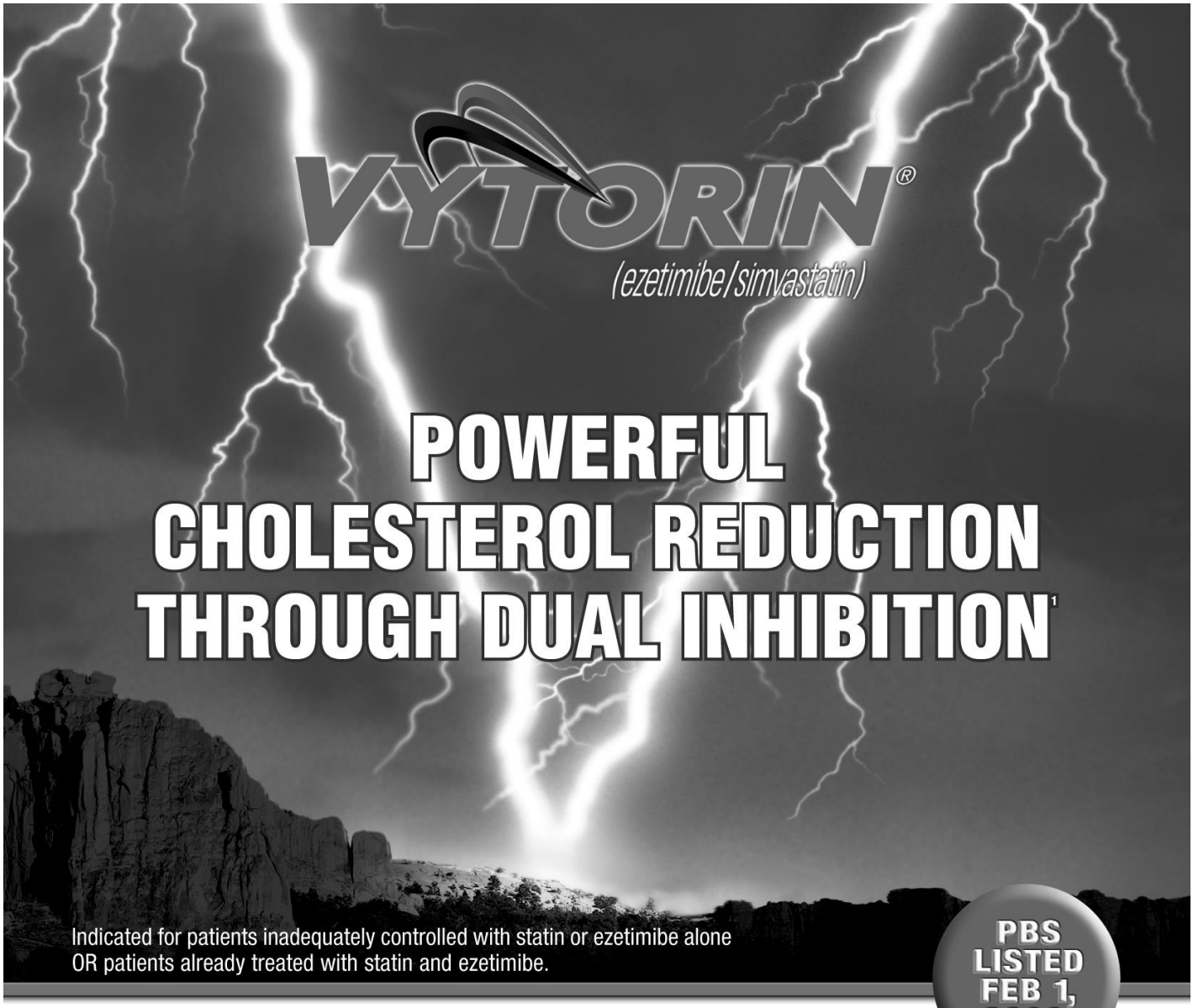

PLEASE REVIEW PRODUCT INFORMATION BEFORE PRESCRIBING. APPROVED PRODUCT INFORMATION AND STARTING PATIENTS INFORMATION IS AVAILABLE FROM MERCK SHARP & DOHME. **Indications:** as adjunctive therapy to diet in patients with primary hypercholesterolaemia or mixed hyperlipidaemia where use of a combination product is appropriate. Patients with homozygous familial hypercholesterolaemia. **Precautions:** myopathy/ rhabdomyolysis, particularly if co-administered with itraconazole, ketoconazole, erythromycin, clarithromycin, HIV protease inhibitors, nefazodone, cyclosporin, danazol, niacin (≥1g/day), amiodarone, verapamil, diltiazem; liver enzymes; high alcohol use; hepatic insufficiency; co-administration with fibrates; monitor prothrombin time if used with coumarin derivatives. **Contraindications:** hypersensitivity; active liver disease; unexplained persistent elevations of serum transaminases; myopathy secondary to other lipid-lowering agents; pregnancy; lactation. **Pregnancy:** Category D. **Adverse events:** dizziness; headache; arthralgia; myalgia; asthenia; abdominal pain; diarrhoea; fatigue; influenza-like illness; muscle cramp; elevations of ALT and/or AST. Adverse events reported rarely include: thrombocytopaenia; cholelithiasis, cholecystitis, hepatitis; arthralgia, myopathy, rhabdomyolysis; hypersensitivity reactions; anaphylaxis, angiodoema; pancreatitis; nausea; increased CPK; elevations of liver transaminases; GI upsets. **Dosage:** Primary hypercholesterolaemia: usual starting dose 10/40mg/day, in the evening, with or without food. Dosage adjustment after 2 weeks if required. *HoFH:* 10/40mg/day or 10/80mg/day in the evening. **Overdosage:** symptomatic and supportive measures should be employed. **Price:** VYTORIN 10/40mg: $135.22; VYTORIN 10/80mg: $163.01. VYTORIN 10/10mg is not available in Australia. VYTORIN 10/20mg is not PBS listed. References: **1.** Approved Product Information for VYTORIN. ®VYTORIN is a registered trademark of MSP Singapore Company, LLC. 04-07-VYT-06-MSP-198-J. H&T MSD0481/CSANZ

PBS Information: Authority required. Initial treatment for patients with CHD, PVD, familial hypercholesterolaemia or diabetes mellitus whose cholesterol levels are inadequately controlled after ≥3 months treatment with ≥40mg statin. Pathology lab results must be ≤1 month old. Continuing treatment for patients previously issued with a PBS VYTORIN prescription or ezetimibe + ≥40mg statin. Refer to PBS Schedule for full authority requirement information.

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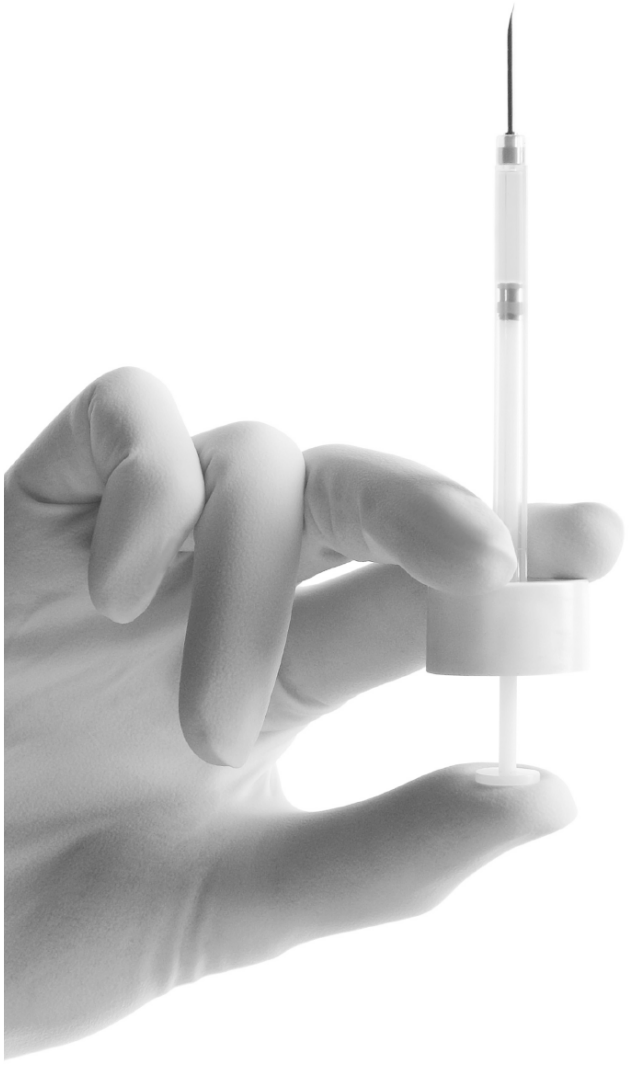

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- Well tolerated by patients with acromegaly or symptoms of carcinoid syndrome[2,3,6–8]
- The only long-acting somatostatin analogue that does not require pre-treatment using a subcutaneous preparation[1,9]

time to simplify.

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1. Somatuline Autogel Product Information, 17 December 2004. 2. Ashwell SG *et al. Eur J Endocrinol* 2004;**150**:473–80. 3. Alexopoulou O *et al. Eur J Endocrinol* 2004;**151**:317–24. 4. van Thiel SW *et al. Eur J Endocrinol* 2004;**150**:489–95. 5. Gutt B. Poster presented at the Endocrine Society 85th Annual Meeting, Philadelphia, June 2003. Poster P3-514. 6. Ruszniewski P *et al. Neuroendocrinology* 2004;**80**:244–51. 7. Caron PH *et al. J Clin Endocrinol Metab* 2002;**87**:99–104. 8. Caron PH *et al. Clin Endocrinol* 2004;**60**:734–40. 9. Sandostatin LAR Product Information, *MIMS Annual*, June 2005.

PBS Information: Authority required (Section 100). Acromegaly; Symptoms of carcinoid syndrome.
Refer to PBS schedule for full information.

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. 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. **Storage**: 2°C–8°C. **Date of TGA approval**: 17 December 2004.

For further information about Somatuline Autogel, contact Ipsen: **T** (03) 8544 8100 **F** (03) 9562 5152 **E** info@ipsen.com.au
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001

THE ROLE OF NON-CODING RNA IN HUMAN DEVELOPMENT

J. S. Mattick

Institute for Molecular Bioscience, University of Queensland, St. Lucia, QLD, Australia

It appears that we have fundamentally misunderstood the nature of genetic programming in humans and other multicellular organisms for the past 50 years because of the presumption, largely correct in prokaryotes but not in complex eukaryotes, that most genetic information is transacted by proteins, which form the main analog components of all cells. Humans have the same number of protein coding genes (19,500) as the nematode worm (~19,300), which has only 1,000 cells. Although only 1.2% of the human genome encodes proteins, the vast majority is actually transcribed in a developmentally regulated fashion, much of it on both strands. These transcripts include tens if not hundreds of thousands of small RNAs, including miRNAs, snoRNAs, piRNAs and other yet-to-be-discovered classes of regulatory RNAs, many of which are encoded in introns, and longer noncoding RNAs that exhibit dynamic expression patterns during germ cell and ES cell differentiation, gonadal development, muscle development, brain development, and macrophage and T-cell activation, to name a few. Many are dysregulated in disease, including neurological diseases and cancer. It is also now evident that most, if not all, complex genetic phenomena in the higher organisms are directed by RNA signaling pathways. Taken together, the data suggest that most of the human genome and those of other complex organisms, including transposon-derived sequences, is not junk nor evolving neutrally, but rather encodes a hitherto hidden layer of regulatory RNAs (many of which are species- or lineage-specific) that set the settings and direct the trajectories of differentiation and development via the control of chromatin architecture and epigenetic memory, promoter selection, splicing, RNA modification and editing, and mRNA stability and translation.

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002

WHY MEN MAKE SPERM AND WOMEN MAKE OOCYTES: DISCOVERY OF THE MOLECULAR SIGNALS CONTROLLING GERM CELL FATE DURING EMBRYONIC DEVELOPMENT

J. Bowles[1], D. Knight[1], C. Smith[1], M. J. Wilson[1], D. Wilhelm[1], J. Richman[1,2], S. Mamiya[3], K. Yashiro[3], K. Chawengsaksophak[4], J. Rossant[4], H. Hamada[3], P. Koopman[1]

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[2]Department of Oral Health Science, Faculty of Dentistry, University of British Columbia, Vancouver, Canada
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[4]The Hospital for Sick Children, Toronto, Ontario, Canada

In mouse embryos, germ cells in developing ovaries enter meiosis and begin oogenesis around 13.5 dpc, whereas those in male gonads cease dividing until after birth, signaling a spermatogenic fate. It is widely believed that germ cells are intrinsically programmed to enter meiosis at a predetermined time, unless prevented from doing so by factors secreted by the male gonad. Instead, we now find that retinoic acid (RA) signaling controls the nexus between spermatogenesis and oogenesis.

We conducted an expression screen designed to identify genes expressed in a male- or female-specific manner during mouse gonadogenesis, and identified two genes encoding enzymes involved in RA metabolism. We detected abundant RA production in the adjacent mesonephroi of both sexes; the RA diffuses into the gonads in both sexes, persisting at high levels in the ovary, but is cleared from developing male gonads by the degradative enzyme Cyp26B1. By treatment of gonadal explants, we showed that either retinoic acid or an inhibitor of CYP26B1 induces XY germ cells to enter meiosis. Conversely, a retinoic acid receptor antagonist blocks entry of XX germ cells into meiosis. Meiotic markers were also induced in testes of *Cyp26b1* knockout mice. Together, our data suggest that retinoic acid, produced by the mesonephros, induces germ cells in the female gonad to enter meiosis but is prevented from doing so in the male gonad because of the actions of CYP26B1. Our data identify RA as a molecular trigger of meiosis in fetal gonads, a discovery that may be applicable to modulating human or animal fertility *in vivo* and production of functional gametes from germline stem cells *in vitro*.

003

A NOVEL APPROACH TO STUDY HUMAN PROSTATE DEVELOPMENT AND ITS DISEASES

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Centre for Urological Research (CURe), Monash Institute of Medical Research, Monash University, VIC, Australia

Men are not mice and rodent models have limited utility and relevance when studying human diseases. This is particularly true in the study of prostate disease, both benign and malignant, since these do not occur in mice. Yet mice are commonly used for this purpose and rodent models have provided controversial evidence for the early origins of adult prostate disease that is almost impossible to verify in humans. How do we develop model systems of human disease?

Our approach was to use the classical biological technique of tissue recombination together with stem cell technology to generate non-diseased human prostate tissue. We used rodent mesenchyme to establish reciprocal stromal-epithelial cell interactions with human ESC and directed their differentiation to fetal and mature human prostate glands, expressing PSA (prostate specific antigen), within 12 weeks; a process that takes 15 years or more in men. Glands derived from hESC of different genetic sex, first express fetal markers of prostate differentiation (eg Nkx3.1), followed by markers of its maturation (eg AR, p63 and PSA) and all the tissues are hormonally responsive.

This model provides new opportunities to study prostate disease. Firstly it provides normal tissue that is only available from young men aged 20-30yo; from this normal tissue, the process of disease initiation and progression can be studied, especially cancer. The mechanism of disease induction can be explored and verified by up or down regulating specific gene expression in the mesenchyme or stroma, eg using genetically modified mouse tissue or siRNA. Further, since fetal tissues are generated, we are able to study the early origins of adult disease, specifically the transgenerational effects of endocrine disruptors.

The conservation of stromal-epithelial signalling mechanisms between rodent and human species suggests this approach could extend to integumental, gastrointestinal and genital tissues, enabling the development of more relevant models of human diseases.

005

LIFE IN THE POUCH: WOMB WITH A VIEW

M. B. Renfree

Zoology, The University of Melbourne, Melbourne, VIC, Australia

Marsupials give birth to altricial young after a relatively short gestation period, but have a long and sophisticated lactation while the young develop, usually within a pouch. Their viviparous mode of reproduction thus minimises placentation in favour of lactation, effectively trading the umbilical cord for the teat. The special adaptations that marsupials have developed provide us with unique insights into the evolution of mammalian reproduction. Marsupials hold many mammalian reproductive "records", for example they have the shortest known gestation but the longest embryonic diapause; the smallest neonate but the longest sperm. They have contributed to our knowledge of many mammalian reproductive events including embryonic diapause and development, birth behaviour, sex determination, sexual differentiation, lactation and seasonal breeding. Since marsupials have been genetically isolated from eutherian mammals for over 125 million years, sequencing of the genome of two marsupial species has made comparative genomics an exciting and important new area of investigation. This review will show how the study of marsupials has widened our understanding of mammalian reproduction and development, highlighting some of the mechanisms that are so fundamental that they are shared by all today's marsupial and eutherian mammals.

007

THE PIVOTAL ROLE OF IGF-II IN PLACENTAL INVASION, GROWTH AND FUNCTION

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The placenta has a myriad of functions including exchange of oxygen, nutrients and wastes between the maternal and fetal circulations. In early pregnancy, placental trophoblast cells invade and colonise the decidua and its vasculature to sequester a blood supply for the growing placenta. Impairments in this process have been implicated in pregnancy complications including IUGR, preeclampsia and pre-term birth. Our research is elucidating the pivotal role of IGF-II in placental invasion, differentiation and growth. IGF-II promotes trophoblast invasion while TGF β 1 inhibits it. We have discovered that IGF-II, under the influence of the low oxygen environment that characterises the first trimester, interacts with the IGF2R and the plasminogen activator system in 7-8 weeks human placental villous explants to promote trophoblast differentiation down the invasive pathway. In addition, treatment of the guinea pig with IGF-I or –II in early to mid pregnancy increases fetal weight near term. IGF-II improves the placental structural capacity for exchange near

term, while IGF-I reduces maternal fat deposition, presumably affecting substrate availability. Both IGFs sustainedly improve placental transport of glucose and amino acids. Hence, the type 1 and 2 IGF receptors are likely to mediate different IGF actions during early pregnancy. Gene ablation studies have shown that, later in gestation, IGF-II plays an important role in placental transport functions. Therefore, factors that reduce placental expression of IGF-II are likely to compromise placental exchange late in gestation when the demands of the fetus escalate. We have also discovered that repeated glucocorticoid treatment on days 104, 111 and 118 of pregnancy in the ewe significantly reduces placental IGF-II and IGF2R mRNA expression at day 145 just before term. This treatment also reduces fetal growth and therefore is likely to impair placental transport functions. Together these data demonstrate that IGF-II is a key player in placental development and function.

008

MACROPHAGE INHIBITORY CYTOKINE-1: ROLES IN TROPHOBLAST FUNCTION AND DECIDUAL PREPARATION

E. M. Wallace[1], B. Marjono[1], D. Brown[2], S. Breit[2], E. Dimitriadis[3], L. Salamonsen[3], U. Manuelpillai[1]

[1]Obstetrics and Gynaecology, Monash University, Clayton, VIC, Australia
[2]Centre for Immunology, St.Vincent's Hospital and University of New South Wales, Sydney, NSW, Australia
[3]Prince Henry's Institute of Medical Research, Clayton, VIC, Australia

Successful placentation is fundamental to the development of a healthy pregnancy and delivery of normal well grown baby. Understanding and manipulating placentation is therefore key to improving outcomes in various pregnancy disorders such as miscarriage, fetal growth restriction and pre-eclampsia. Over recent years, we have been exploring the roles of macrophage inhibitory cytokine-1 (MIC-1), a transforming growth factor-ss superfamily member, in the regulation of placentation, decidualisation and subsequent pregnancy success. We have shown that MIC-1 is localized to the syncytiotrophoblast layer of the placenta and that MIC-1 production is down regulated in invasive extravillous trophoblast cells. Consistent with this, MIC-1 inhibits the activation of matrix metalloproteinases –2 and –9 in first trimester trophoblast and inhibits outgrowth from villous explants. These data suggest that MIC-1 may regulate trophoblast invasion/placentation. MIC-1 is also localized to the endometrium in both glandular and stromal cells with increasing immunostaining in secretory and decidualised tissues. In vitro, MIC-1 secretion by endometrial stromal cells increases during decidualisation and, in turn, MIC-1 facilitates the process of decidualisation. We have also undertaken a number of clinical studies of MIC-1 levels in maternal serum. In asymptomatic women who subsequently miscarry, first trimester MIC-1 levels are profoundly lower than in women with a subsequently normal pregnancy, consistent with MIC-1 having important roles in early pregnancy establishment. These data offer the potential for new clinical diagnostics and therapeutics. In summary, MIC-1 appears to have a number of potentially important functions in the early human placenta and decidua consistent with physiological roles in normal placentation. Whether these functions are key to successful pregnancy and the diagnostic utility of MIC-1 in early pregnancy remain key questions for our group.

009

INTERLEUKIN 11 AND LEUKEMIA INHIBITORY FACTOR: MECHANISMS AND INTERACTIONS IN IMPLANTATION AND PLACENTATION

E. Dimitriadis

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

The successful implantation of the human embryo into a receptive endometrium leads to the formation of a functional placenta. Implantation failure results in infertility, while impaired implantation leads to inadequate placentation. Deficiencies in placental development can result in early abortion, or pre-eclampsia and intrauterine growth restriction. Currently there is no way of diagnosing endometrial infertility in women or of establishing whether the placenta is developing adequately. It is critically important to understand the molecular mechanisms of implantation because deficiencies in implantation have such serious consequences. Endometrial interleukin (IL)-11 and leukemia inhibitory factor (LIF) belong to the IL-6 family of cytokines and are two of very few molecules unequivocally required for embryo implantation and establishment of pregnancy in mice. In humans, IL-11 and LIF are produced by the endometrium and placenta in a spacial and temporal pattern suggestive of roles in uterine receptivity, endometrial stomal cell decidualizaton and trophoblast function. IL-11 advances decidualization of human endometrial stromal cells and LIF enhances endometrial stromal cell survival *in vitro*. However, roles for IL-11 in endometrial epithelial and trophoblast function are unknown, and for LIF, poorly understood. New evidence will be discussed in terms of the roles and interactions of IL-11 and LIF in uterine receptivity. A key issue in placental development is what controls trophoblast invasion during early placental development. Novel roles for IL-11 and LIF in trophoblast invasion will be presented. Studies that highlight the potential use of IL-11 and LIF as targets of infertility will also be discussed.

010

DETERMINANTS OF TISSUE AND LIGAND SELECTIVITY IN THE MINERALOCORTICOID RECEPTOR

P. J. Fuller, V. Yao, F. M. Rogerson

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

The mineralocorticoid receptor (MR) differs from the other steroid receptors in that it responds to two physiological ligands, aldosterone and cortisol (1). In epithelial tissues, aldosterone selectivity is determined by the activity of 11β 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. Clinical trials demonstrate a benefit of MR antagonists in the treatment of cardiac failure, however this benefit is compromised by hyperkalaemia. There is thus a need to search for tissue and ligand-specific determinants of MR activation.

Using a chimeric approach (2), we exploited the inability of the GR to bind aldosterone to identify the region of the MR ligand-binding domain (LBD) that confers aldosterone binding. We have narrowed this to a region of 25 amino acids, curiously the residues in this region that permit aldosterone binding do not contribute to the ligand-binding pocket.

Although the steroid receptors are modular, interactions may occur between domains. The N/C-interaction (3) is aldosterone-dependent but unexpectedly cortisol is an antagonist.

Nuclear receptor mediated transactivation is critically dependent on, and modulated by, co-regulatory molecules. A yeast-2-hybrid kidney cDNA library screen with the MR LBD has identified proteins which interact with one but not both MR ligands.

Further characterisation of these interactions may provide the basis of screens for the identification of "selective mineralocorticoid receptor modulators".

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011

ROLES OF ORPHAN RECEPTOR LRH-1 IN REPRODUCTION AND CANCER

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Liver Receptor Homolgue-1 (LRH-1) is an orphan member of the nuclear receptor superfamily that belongs to the NR5A subgroup of receptors. Originally identified as a liver-specific factor that regulates expression of alphafetoprotein, LRH-1 has now been implicated in a variety of processes including cholesterol and bile acid synthesis, steroidogenesis and embryonic development. We have shown roles for LRH-1 in regulating aromatase expression in adipose tissue, testis and granulosa cells. LRH-1 also appears to mediate the over-expression of aromatase that occurs in adipose tissue of breast cancer patients, thereby providing the source of oestrogens for growth of postmenopausal ER+ tumours. In addition, LRH-1 directly stimulates proliferation of breast cancer epithelial cells by stimulating expression of G_1 cyclins. As such, it is an attractive target for drug development. As an orphan receptor, however, LRH-1 is constitutively active in the absence of ligand, and to date no antagonists have been identified. We are using complementary approaches to identify selective LRH-1 modulators. Firstly, by using phage display we have isolated small peptides that can inhibit LRH-1 activity by preventing its ability to interact with endogenous co-activators. Secondly, *in silico* approaches are being utilised to identify small "drug-like" molecules that either mimic the peptide antagonists by binding to the same site, or else occupy the classical ligand binding site of the receptor. These approaches may produce useful tools to dissect the functions of LRH-1 in endocrine tissues, and also have the potential to realise a new class of nuclear receptor modulators.

012

VITAMIN D-WNT PATHWAY INTERACTIONS IN SKELETAL AND NON-SKELETAL CELLS

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Active hormonal vitamin D, calcitriol, inhibits cell proliferation and induces differentiation and apoptosis in normal and tumor cells. Wnt signaling is involved in embryonic developmental and in adult tissue homeostasis, regulating cell fate specification, proliferation and differentiation. In addition, Wnt pathway dysregulation occurs in tumor cells. Secreted Wnt family glycoproteins act through Frizzled receptors to stimulate canonical β-catenin-mediated transcriptional responses. Evidence that human bone mass is strongly affected by mutations of LRP5, a Frizzled co-receptor, has led to investigation of interactions between Wnt and other bone regulatory pathways, including the vitamin D response

pathway. In addition, study of vitamin D-Wnt pathway interactions has also been stimulated by findings that vitamin D analogues promote differentiation of human colon carcinoma cells, in which β-catenin protein level or activity is often elevated. Calcitriol-bound vitamin D receptor (VDR) can directly inhibit the Wnt response by interaction with β-catenin, sequestering it away from the Wnt-responsive TCF transcription factor complex. As a result, activation of vitamin D responsive promoters is potentiated while transcriptional regulation of Wnt target genes is reduced. These divergent transcriptional effects are due to direct interaction between the β-catenin C-terminus and the VDR activation function-2 domain, with acetylation at the β-catenin C terminus differentially regulating the transcriptional responses[1]. Calcitriol can also indirectly affect canonical Wnt pathway activity in osteoblasts by enhancing LRP5 gene transcription[2] and in colon carcinoma cells by stimulating expression of E-cadherin, which depletes nuclear β-catenin by promoting its accumulation at the plasma membrane[3]. The relative significance of these direct and indirect interaction mechanisms appears to be dependent at least in part upon cell type and the level of β-catenin present.

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013

NUCLEAR RECEPTORS IN METABOLISM: THE SKI PHENOTYPES AND THE NORPHANS

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Nuclear Receptors (NR) are ligand-activated transcription factors that play key roles in growth, development and metabolism. The NR gene superfamily comprise the steroid receptors (estrogen, androgen, glucocorticoids amongst others), retinoid x-receptor (RXR) heterodimers (including thyroid, retinoic acid, and PPARs) and a large orphan receptor sub-family of receptors with no known ligands (NOR-1/Nurr77, COUPTF, LXR and many others). NRs expressed in skeletal muscle, fat and liver act as nutritional sensors to regulate metabolic target gene transcription to maintain energy homeostasis through direct effects on lipid, glucose and energy metabolism. As such, NRs involved in regulation of metabolism are primary targets for pharmacotherapeutic intervention to prevent and treat diabetes, cardiovascular disease and the metabolic syndrome. NRs being essential for normal growth and development interact with many other signalling pathways, including the transforming growth-factor- β (TGF- β) pathway, to amplify the diversity and complexity of their physiological control on growth. The Ski proto-oncogene, a negative regulator of the TGF- β signalling, recruits corepressors or prevents coactivator recruitment to the active transcriptional complex to determine the ultimate transcriptional outcome and the anti-proliferative potential. Ski *in vivo* and *in vitro* modulates skeletal muscle metabolism in part through an interaction and via regulation of NR-dependent effects on lipogenesis and metabolism. In this seminar, the functional role of several orphan nuclear receptors in skeletal muscle metabolism will be presented, in addition to the role of Ski crosstalk with the NR signalling pathway involved in development of the metabolic syndrome.

014

ASPECTS IN THE DIAGNOSIS AND MANAGEMENT OF PROLACTINOMA

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Human prolactin was isolated in the 1970's and it was soon recognised that hyperprolactinaemia resulted in a syndrome of amenorrhoea/galactorrhoea. Subsequently, it has been shown that hyperprolactinaemia may be the cause of secondary amenorrhoea in up to one third of cases. Prolactinomas are the commonest form of pituitary adenoma, and make up approximately 30% of all pituitary neoplasms. The basic principles of investigation involve excluding physiological and non-neoplastic causes of hyperprolactinaemia, pituitary neuroimaging and biochemical assessment of pituitary function. The recognised indications for treating hyperprolactinemia include hypogonadism (oligo-amenorrhoea in women, androgen deficiency in men), significant symptomatic galactorrhoea and tumour mass effect, particularly where visual pathways are compromised. Where hyperprolactinaemia is asymptomatic, no specific treatment other than periodic observation may be required. Once a prolactinoma is diagnosed, the usual first line of treatment is with a dopamine agonist. Currently, the dopamine 2 receptor specific agonist Cabergoline is the most widely used agent in clinical practice. Delivered once or twice weekly, it normalises prolactin levels in over 90% of subjects with pathological hyperprolactinaemia. The usual dose range is from 0.5 to 3mg per week, but higher doses may be used in resistant cases. Published data regarding macroadenoma shrinkage are uncontrolled but also demonstrate equal or superior efficacy compared to older studies of Bromocriptine, and occurs in approximately 80% of patients. Recent evidence suggests that over 60% of cases treated for 3-4 years with Cabergoline may enter a long term remission on cessation of the drug. Resolution of the adenoma on MRI is predictive of remission. Surgery is generally

reserved for a) dopamine-agonist resistant tumours, b) adverse effects of dopamine agonists or c) where it is desirable to obtain a histological diagnosis. Management of prolactinoma during pregnancy will be briefly discussed. Historically, Bromocriptine has been preferred although there is no evidence that Cabergoline is associated with an increased rate of foetal anomalies.

015

ACROMEGALY: NEW FRONTIERS IN MANAGEMENT

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Over the last few decades, there have been important advances in the fields of neuroendocrinology, cell biology, clinical chemistry, drug development, imaging, neurosurgery and radiotherapy, all of which have had a major impact in the management of acromegaly. The merits of growth hormone (GH) and insulin-like growth factor (IGF)-I measurements in the diagnosis and in the assessment of therapeutic outcomes of acromegaly have been intensively studied. The biochemical targets for treatment are a growth hormone of <2.5 ng/mL and a normal, age-adjusted insulin-like growth factor-1. Until 20 years ago, dopamine agonists were the only class of pharmaceutical agents available to control acromegaly. They have a limited adjunctive role, even with the development of second-generation selective agonists such as cabergoline. Surgery and radiotherapy were the mainstay of acromegaly management before the advent of the effective pharmacological therapies of the modern era: somatostatin analogues and pegvisomant, a growth hormone receptor antagonist. Somatostatin analogues achieve biochemical control in approximately 60% of patients. Pegvisomant, which is available in the USA and Europe and has just been registered in Australia, normalizes IGF-1 in nearly all patients but has no effect on tumour mass. Surgery is an appropriate first-line therapy for microadenomas as the chance of success is high. For large and/or invasive tumours where the prospect of surgical cure is remote, first-line therapy is somatostatin analogue treatment with debulking surgery having an adjunctive role to achieve tight control or to alleviate compression of the optic chiasm. Although acromegaly remains a challenging disease to manage, the expanding range of therapeutic options is likely to result in a better outcome for patients and offers the potential to tailor therapy based on a patient's individual requirements.

017

INSIGHTS INTO MONITORING THERAPY, AND CHALLENGES IN MANAGEMENT OF HYPOPITUITARY PATIENTS

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Pituitary hormone secretion is complex, dynamic and responsive to multiple external stimuli. This makes replication of normal hormone function in the hypopituitary patient very challenging. Evaluation of possible deficiencies of pituitary hormone secretion is the first challenge the clinician must face. The utility of different static and dynamic tests will be discussed. Replacement therapy with thyroid hormones and gonadal steroids are relatively straightforward, although the route of administration and dosage of sex hormones need to be optimized for each individual. More problematic is determination of the appropriate dosage and formulation for glucocorticoid replacement. We lack any effective method for measuring glucocorticoid action on target tissues, and measurements of circulating adrenal steroids are complicated by diurnal rhythms and variation in binding protiens. Possible approaches to monitoring of cortisol levels and action in ambulatory patients will be discussed. Even greater controversy surrounds the issues of Growth Hormone replacement in GH deficient adults, and androgen therapy in women with primary or secondary hypoadrenalism. Available evidence will be reviewed and balanced with practical aspects of cost and availability of appropriate therapeutic agents in Australia. Finally, some difficulties in management of diabetes insipidus will also be discussed. Patients with hypopituitarism have complex problems, and many remain symptomatic despite apparent “normalization” of their hormone concentrations. A flexible and individualized approach is important in achieving the best possible outcome for each patient.

018

NUTRACEUTICAL AND PHARMACEUTICAL EFFECTS ON UTERINE AND HORMONAL RESPONSES ASSOCIATED WITH EARLY PREGNANCY IN LACTATING DAIRY CATTLE

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A timed insemination program was used to investigate mechanisms through which polyunsaturated fatty acids (PUFA) and bovine somatotropin (bST) may increase fertility. Cows were assigned randomly to be inseminated (d 0) or not inseminated, and to receive 0 or 500 mg of bST (at d 0 and d 11) (i.e., C [cyclic], bST-C, P [pregnant], bST-P). Furthermore, a fish oil-enriched lipid supplement (FO; 1.9% of dietary DM initiated at 10 DIM) was evaluated in cyclic cows with (bST-FO; bST-C) and without (FO; C) bST. On d 17 (~ 94 d DIM) cows were slaughtered, uteri flushed and endometrial tissue collected. BST increased milk production, pregnancy rate (83% [5/6] > 40% [4/10]), conceptus length (45 > 34 cm) and interferon-τ in uterine luminal flushings (9.4 > 5.3 mg) with no effect on interferon-τ mRNA concentration in the conceptus. Feeding FO to cyclic cows increased proportions of eicosapentaenoic and docosahexaenoic acids while reducing the proportion of arachadonic acid in the endometrium. Cyclic cows fed FO had lower plasma insulin than control-fed cyclic cows, and FO altered plasma GH (bST-FO > bST-C) and IGF-I (bST-C > bST-FO) responses to bST. Endometrial IGF-I mRNA was reduced in pregnant cows. IGF-II mRNA was increased in the endometrium of P and bST-treated cows fed the control diet. Cows fed FO had increased concentrations of IGF-II mRNA when bST was not injected. IGFBP-2 mRNA was increased in bST-P cows, whereas bST decreased IGFBP-2 mRNA in all cyclic cows. FO decreased FGF-2 and increased progesterone receptor (PR) mRNAs. BST increased PR mRNA in endometrium of C but not in FO-fed or P cows. Concentrations of ERα mRNA and protein, and oxytocin receptor mRNA were decreased in P compared to C cows. Immunohistochemistry indicated that P and FO decreased ERα abundance in luminal epithelium. PGHS-2 protein was elevated in P cows and localized to the luminal epithelium. Both FO and bST treatments reduced staining intensity of PGHS-2 protein. In summary, pregnancy and bST altered endometrial gene expression. Cyclic cows responded differently to bST than pregnant cows. Feeding FO modulated responses in a manner that may favor maintenance of pregnancy.

019

IODINE SUFFICIENCY ACROSS AUSTRALIA: DO WE CURRENTLY MAKE THE GRADE?

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Over the past 7 years several localised, regional studies in South Eastern Australia and Tasmania have documented the re-emergence of mild to moderate iodine deficiency in adults and children. To provide a comprehensive snapshot of iodine nutrition throughout Australia we undertook a National Iodine Nutrition Study between mid 2003 and end 2004.

Design and Setting:

The survey was a cross-sectional study of 8 to 10 year old school children, randomly selected from government and non-government primary schools, in the 5 mainland Australian states of New South Wales, Victoria, South Australia, Western Australia and Queensland. The sample consisted of 1,709 students from 88 schools, comprising 881 boys and 828 girls. 1) Urinary iodine excretion levels (UIE) were determined and compared with WHO/ICCIDD criteria for the severity of iodine deficiency. 2) Thyroid volumes measured by ultrasound were compared with new international reference values (WHO/ICCIDD).

Results:

On a State basis, NSW and Victorian children are mildly iodine deficient with median UIE levels of 89μg/L and 73.5μg/L, respectively. South Australian children are borderline iodine deficient with a median UIE of 101μg/L. Both Queensland and Western Australian children are iodine sufficient with median UIE levels of 136.5 μg/L and 142.5 μg/L, respectively. Ongoing studies in NSW of iodine nutrition in pregnant women, and their offspring, confirm mild to moderate iodine deficiency is widespread throughout the State.

Conclusion:

The results of this study confirm the existence of inadequate iodine intake in the Australian population and call for the implementation of mandatory iodisation of all edible salt in Australia. In the interim, we recommend iodine supplementation be considered for pregnant women, those contemplating a pregnancy, and breastfeeding mothers.

020

SEARCHING FOR STEM CELLS IN THE KIDNEY

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The kidney, unlike the liver, never undergoes a structural repair or regenerative process in response to damage. Our understanding of kidney development suggests that nephron endowment is finalized prior to birth and that while they can hypertrophy, no new nephrons are 'born' after birth. However, recent data, particularly in the brain but also in other postnatal tissues including the heart and adipose, suggests that there may be stem cells present in adult organs previously regarded as non-proliferative during the postnatal period. We have taken several approaches to investigate the possibility of adult stem cells in the murine kidney. The first involved defining markers of renal progenitors by examining the expression profiling of the intermediate mesoderm as it commits to a renal fate. We have also thoroughly characterised the expression profile, multipotency and renal lineage potential of a putative stem cell population in the kidney, the Hoescht-effluxing side population. In this presentation, we will discuss the results of these studies and reflect on what implications these might have in the development of novel treatments for renal failure.

021

IDENTIFICATION OF MAMMARY STEM CELLS AND THEIR ROLE IN BREAST CANCER.

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The identity and purification of mammary stem cells (MaSCs) has proved elusive due to the lack of defined markers. However, we have recently isolated discrete populations of mouse mammary cells on the basis of cell-surface markers and identified a subpopulation (Lin-CD29hiCD24+) that is highly enriched for MaSCs as assayed by *in vivo* transplantation. Indeed, we demonstrated that a single cell, marked with a *lacZ* transgene, could reconstitute a complete mammary gland *in vivo*. The transplanted cell contributed to all three mammary epithelial lineages and and extensive lobuloalveolar units were generated during pregnancy. Serial transplantation revealed that these cells have self-renewing capacity. These data establish that single cells within the Lin-CD29hiCD24+ population have the multipotent and self-renewing properties that define the MaSC. To further characterise the different mammary epithelial populations, we have investigated the expression of important prognostic markers of human breast cancer, including the estrogen receptor α (ERα), and progesterone receptor (PR), and provide evidence for differential expression amongst the various subsets. Finally, we show that the stem cell population was expanded in premalignant mammary tissue from MMTV-*wnt-1* but not MMTV-*neu* transgenic mice, indicating that stem cells are the likely tumour-initiating cells the *wnt-1* model of breast tumorigenesis.

022

GERMLINE STEM CELLS IN THE OVARY

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The notion of a fixed, non-renewable pool of oocytes in the ovary around birth (1) has been questioned (2,3,4). The presence of germline stem cells (GSC) giving rise to new oocytes in the adult ovary has been proposed (3,4) but challenged on theoretical and methodological grounds (5,6). Two reports provide new data that inform this debate (2,6). Byskov et al (6) injected 30-day old C57BL/6 mice with BrdU and could not find labeled oocytes in primordial or later stage follicles 8 days later. This argues against the presence of mitotically-active GSC in the mouse ovary, but does not exclude differentiated GSC arising from within the ovary or from external sources. We quantified all healthy follicles in C57BL/6 mouse ovaries between days 1 and 200 (n=6-10) using unbiased stereological methods (2). Mean numbers of healthy follicles fell 60% between days 1 and 7, primarily due to expulsion from the ovary. Although we saw no evidence for GSC, rare mitotic figures in unidentified cells were noted between days 1 and 12. From day 7 to 100 mean numbers of primordial or total follicles per ovary were not significantly depleted, but declined by day 200 to about 10% of day 1 levels. Our data supports the hypothesis of follicle renewal in postnatal and adult ovaries of C57BL/6 mice by an as yet unknown mechanism.

Supported by the NH&MRC (#198705 & #241000)

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023

ADULT STEM/PROGENITOR CELLS IN THE ENDOMETRIUM

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The human endometrium undergoes regeneration, differentiation and regression with each menstrual cycle, following parturition and in post-menopausal women taking estrogen replacement therapy. In other regenerative tissues, rare populations of adult stem cells have been identified. While it was postulated many years ago that endometrial stem/progenitor cells were responsible for endometrial regeneration, it was not until recently that we provided the first evidence for their existence in human and mouse endometrium, setting a new paradigm in uterine biology (1,2). We demonstrated that 0.22% of endometrial epithelial cells and 1.25% of stromal cells were clonogenic, each producing two morphologically distinct colony types (1). The large clones (colony forming units, CFU) of small, densely packed cells were rare (0.09% of epithelial and 0.02% of stromal), and the small clones comprised large, loosely arranged cells. Large epithelial and stromal CFU exhibited several adult stem cell properties; high proliferative potential and self-renewal. Large stromal CFU cultured in appropriate induction media also underwent multilineage differentiation into mesenchymal lineages; fat, smooth muscle, bone and cartilage. Bone marrow mesenchymal stem cell (MSC) phenotypic markers CD29, CD44, CD90, CD73, CD105, CD146 were expressed by large stromal CFU. Epithelial (2.1%) and stromal (0.2%) cells showed side population (SP) phenotype (stem cell property). Co-expression of CD146 and PDGF receptor-β produced a 10-fold purification of stromal cells with CFU and multilineage differentiation capacity. These cells were located around blood vessels. Our studies in mouse endometrium using the label retaining cell (LRC) technique identified the location of candidate endometrial epithelial stem/progenitor cells in the surface epithelium and MSC-like cells at the endometrial-myometrial junction (2). These rare epithelial and stromal LRC rapidly proliferated on estrogen-induced endometrial regeneration, despite lack of estrogen receptor-α expression. Our data suggest that rare populations of epithelial progenitors and MSC-like cells exist in human and mouse endometrium and may be responsible for initiating endometrial regeneration.

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024

NEW GENES THAT CONTROL REPRODUCTION IN THE HUMAN AND THEIR GENOTYPE-PHENOTYPES: EVIDENCE FROM HUMAN DISEASE MODELS

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In all mammalian species, gonadotropin-releasing hormone (GnRH) represents the key interface between the way that the CNS views the outside world and how it transmits signals internally to its reproductive endocrine milieu. Since it is the first and initiating hormone in a complex reproductive cascade that involves gonadotropin biosynthesis and secretion, gonadal steroidogenesis and germ cell maturation, and behavoir, it can be viewed as the "pilot light of reproduction".

Therefore, understanding the genes and signals that modulate the developmental fate and secretory actions of GnRH neurons in man remains a major question as defined in Science Magazine's 125 Outstanding Scientific Questions in 2005. To gain insight into this problem, we have used the human disease models of normosmic idiopathic hypogonadotropic hypogonadism (nIHH) and Kallmann Syndrome (KS) to elucidate the genes that control GnRH's secretion and action. Since patients with these conditions represent isolated defects in the secretion or action of GnRH, understanding their genetic basis has proven to be an important avenue of biologic insights into this problem.

This lecture will report on several new genes that are responsible for control of this key reproductive peptide in the human that we have identified using these human disease models, including GPR54, Metastin, FGFR1, and GnRHR. It will review new mutations in each and their genotype/phenotype correlations as well as useful clinical points for their counseling and management.

025

KISSPEPTIN ACTIVATION OF GNRH NEURONS TO INITIATE PUBERTY AND OVULATION.

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Evidence is accumulating for a critical role of kisspeptin signaling within the neural circuitry controling fertility. Our studies have focussed upon understanding the cellular mechanisms through which kisspeptin controls the activity of the gonadotropin-releasing hormone (GnRH) neurons in mice.

Immunocytochemical approaches have demonstrated that a large population of kisspeptin neurons located in the rostral periventricular region of the hypothalamus develop just prior to puberty. Studies using a Cre-dependent Pseudorabies virus retrograde tracing technique in transgenic GnRH-Cre mice have shown that a sub-population of periventricular kisspeptin neurons project directly to GnRH neurons. Across development, GnRH neurons can be seen to receive kisspeptin-immunoreactive fibre appositions from postnatal day 25 onwards and electrophysiological studies in GnRH-GFP mice show that the percentage of GnRH neurons responding directly to kisspeptin increases across puberty. These studies suggest that periventricular kisspeptin neurons innervate and activate GnRH neurons directly to help bring about puberty.

Estrogen positive feedback initiates ovulation through a neural pathway that involves sexually dimorphic, estrogen receptor alpha (ERα)-expressing neurons that activate GnRH neurons. Our recent studies have shown that periventricular kisspeptin neurons in the mouse (1) are sexually differentiated with females having 10-fold great numbers of kisspeptin neurons compared with males, (2) express ERα, and (3) are activated by estrogen positive feedback using estrogen or ERα-selective agonists. Viral retrograde tracing shows that ERα-expressing kisspeptin neurons are primary afferents to GnRH neurons. Coupled with electrophysiological evidence for a massive and prolonged excitatory action of kisspeptin on GnRH neurons at proestrus, these data strongly suggest that periventricular kisspeptin neurons are a key component of the neural mechanism initiating ovulation.

026

THE BIOLOGY OF KISSPEPTINS AND GPR54

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In July 2005, Science magazine listed as one of its 125 greatest unanswered scientific questions "What controls puberty"? The power of genetics resulted in the discovery of the GPR54/Metastin system in the human by 2 groups in 2003 (DeRoux et al; Seminara et al). Since then, it has become clear that this system, previously completely overlooked by basic investigators attempting to determine the long-elusive "puberty gene", actully fulfills the criteria for a ligand receptor system that serves as a major gatekeeper of puberty and sexual maturation in several species including the human, mouse, and monkey. GPR54 receptors are located in the medial basal hypothalamus with >75% of GFP labelled GnRH neurons demonstrating their presence. GPR54 levels increase at puberty in both sexes in rodents and monkey. Their neuroanatomic localization in the arcuate nucleus and the AVPV, both important and known sites for the neuroendocrine control of GnRH secretion, position them well to be major regulators of GnRH secretion during puberty. GPR54 levels also appear to be sex steroid responsive although our understanding of the transcriptional control of GPR54 is still in its infancy. The precursor 145 aa peptide ligand for GPR54, Kisspeptin, is cleaved at a dibasic cleavage site and gives rise to an amidated 54 aa carboxy terminal fragment termed Metastin because of its previously described role in limited metastatic disease in several malignant cell lines. It appears that all biologic activity resides in the amidated carboxyterminal decapeptide sequence of Kisspeptin/Metastin although the circulating form of this precursor molecule is not yet clear. Metastin has been determined to be the most sensitive stimulator of GnRH-induced LH secretion yet discovered with levels as low as pM being able to stimulate GnRH secretion in rodents and primates. Metastin administration also causes GnRH antagonist-blockade of LH release (indicating the essential role of GnRH secretion and action in the ensuing LH release). Circulating levels of metastin are detectable in the human and increase 10,000 fold during pregnancy in a pattern that is quite different from hCG. This lecture will focus upon this sytem and its relationship with GnRH secretion.

027

KISSPEPTIN AND GPR54: MOLECULAR CONDUITS FOR PUBERTY ONSET AND CENTRAL INTEGRATION OF ENERGY BALANCE AND REPRODUCTION

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Based on the observation that inactivation of the G protein-coupled receptor GPR54 is linked to hypogonadotropic hypogonadism in humans and mice, the essential role of this receptor and its putative ligands (kisspeptins, encoded by KiSS-1 gene) in the control of reproduction was first proposed in late 2003. Indeed, such a contention has now been fully substantiated by a number of genetic, molecular, physiologic and pharmacological studies. We will review herein the available evidence for the key role of KiSS-1/GPR54 system in the timing of puberty and signaling of energy balance and metabolic information onto the centers governing reproductive function. Concerning puberty onset, hypothalamic expression of KiSS-1 and GPR54 genes has been proven developmentally regulated, with maximum levels at puberty in rodents and primates. Moreover, functional studies have disclosed that enhanced KiSS-1 function (through increased kisspeptin tone and signaling efficiency) takes place at the time of puberty. Nonetheless, pubertal activation of KiSS-1 system appears to be exquisitely modulated, as excessive enhancement of KiSS-1 tone at puberty evokes the 'paradoxical' suppression of the gonadotropic axis. Regarding integration of energy status and reproduction, functional and expression analyses have demonstrated that situations of negative energy balance, linked to hypogonadotropism, decrease the hypothalamic expression of KiSS-1 gene, while exogenous kisspeptin is able to normalize acute gonadotropin responses and restore pubertal activation of the reproductive axis in undernutrition. Similar observations have been obtained in models of altered metabolism and gonadotropin secretion, such as experimental diabetes, where decreased KiSS-1 expression was associated to defective leptin levels, in line with recent data showing reduced KiSS-1 mRNA levels in ob/ob mice. Altogether, these data evidence that, among other essential roles in reproduction, the hypothalamic KiSS-1/GPR54 system operates as pivotal molecular conduit in the timing of puberty onset and for relaying metabolic information onto the centers governing the gonadotropic axis.

028

THE ROLE OF KISSPEPTIN IN MEDIATING SEX STEROID FEEDBACK CONTROL OF GNRH

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The Kiss1 gene encodes a family of peptides called kisspeptins. These peptides are the endogenous ligands for the G protein-coupled receptor, GPR54, and play a vital role in the regulation of GnRH and in turn gonadotrophin secretion. In many species, centrally administered kisspeptin stimulates gonadotrophin secretion in a GnRH dependant manner. Moreover, virtually all GnRH neurons co-express GPR54. In the hypothalamus, the vast majority of kisspeptin producing cells (those expressing KiSS-1 mRNA) also express sex steroid receptors, particularly oestrogen receptor alpha. Thus, sex steroids are able to directly regulate the expression of KiSS-1 mRNA, implicating kisspeptin as a link between sex steroids and GnRH feedback. In the arcuate nucleus (Arc) of the rodent, sex steroids inhibit the expression of KiSS-1 mRNA, suggesting that the kisspeptin secreting neurons here are the conduit for the negative feedback regulation of GnRH/gonadotrophin secretion. However, in the anteroventral periventricular nucleus (AVPV), sex steroids induce the expression of KiSS-1 mRNA, implying that these kisspeptin neurons play a role in the positive feedback regulation of GnRH/gonadotrophin secretion. Thus, it is conceivable that kisspeptin neurons in the AVPV are central processors for generating the preovulatory luteinising hormone surge in the female.

029

LABORATORY MEASURES IN CLINICAL ENDOCRINE PRACTICE: BONE MARKERS

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Current investigations for patients with osteoporosis focus on assessment of bone mass with densitometry. However, clinicians commonly order laboratory tests to identify secondary causes of osteoporosis as well as monitor response to therapeutic interventions. The use of bone markers has been extensively studied and these markers reflect generalised skeletal remodelling. Bone markers may thus offer diagnostic utiltity, prognostic information and represent a useful tool for therapeutic monitoring. Bone markers are classified as either bone formation or bone resorption markers depending on which remodelling process they mainly represent. In most instances both remodelling processes are balanced and either bone marker will reflect the degree of bone remodelling activity. However, because bone resorption is shorter than formation, resorption markers respond faster to changes in remodelling than formation markers. A number of cases will be presented that highlight the current utility as well as limitations of bone markers in clinical practice. Specifically, a thorough understanding of preanalytical factors, analytical issues including lack of standardisation and postanalytical interpretation of results in the context of pathological skeletal disorders will be discussed. All of these issues can result in significant variation in results and an understanding is required when

interpreting individual bone marker responses. Bone markers have a limited role in diagnostic stratification, however could have a greater role in prognostic stratification and therapeutic monitoring which may result in routine clinical application. Recent evidence has indicated that pretreatment bone turnover may predict the reduction in nonspine fractures with alendronate therapy. Reduction in bone turnover occurs earlier than bone density changes following risedronate therapy and accounts for more than 50% of the predicted fracture reduction with treatment. Lastly bone markers may have a role in defining persistence with therapy and compliance with dosing recommendations.

031

MACROPROLACTIN

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Diagnosing hyperprolactinemia has always been confounded by the possible presence of macroprolactin which has been dubbed "false hyperprolactinemia". If not properly investigated macroprolactin can lead to diagnostic confusion, invasive and unnecessary investigations. Prolactin can be present in the circulation as the monomer alone (molecular weight (MW) 23,000), or as a variable mixture of monomer, a polymeric complex of between 40 and 60,000 MW and the monomer bound to an immunoglobulin (MW 150-170,000). Both the monomer and the polymeric complex known as big prolactin have been reported to be biologically active. The immunoglobulin bound form is described as big big prolactin (macroprolactin), which has a long half life in the circulation and limited bioactivity. It is important to screen high prolactin levels for the presence of macroprolactin before commencing an investigation for an adenoma. Some prolactin assays do not measure macroprolactin and others show variable ability to determine it's presence. Routine screening of high prolactin levels has to be done with an assay that detects macroprolactin and has been confirmed to be able to measure Prolactin in the presence of PEG. The immunoglobulin bound prolactin is precipitated with 12.5% PEG (w/v) and a recovery of 40% or less of the immunoreactive prolactin in the supernatant was indicative of the presence of macroprolactin. This also can be misleading and reporting only the PEG recovery can lead to some confusion. The presence of a microadenoma cannot be excluded, unless the recovered prolactin levels returned to the normal range after treatment. To add further confusion using gel exclusion chromatography we have found patients with big prolactin levels that would have appeared to be macroprolactinemiac if only the PEG screening assay procedure was used. Many of the confusing patients may be being detected earlier now because of the increased awareness of macroprolactin in hyper prolactinemia. It is important to recognize the limitations of the screening assay as well as the ability of different prolactin assays to detect macroprolactinemia. It is concerning us that we are detecting an increasing number of patients with all three forms of prolactin in the circulation and a number of these may be incorrectly attributed to the presence of macroprolactinemia.

033

NUCLEAR RECEPTOR COREGULATORS – GETTING TO THE HEART OF HORMONE ACTION

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Discovery of the nuclear receptor coregulators, exemplified by SRC-1, has revolutionized our understanding of hormone action, and provided an opportunity to develop new diagnostic and prognostic markers as well as potential new therapeutics for a variety of human diseases. Numerous coregulators (comprising coactivators and corepressors) have been identified in the past few years, and the challenge is to define their molecular mechanisms of action, their functional role in a tissue- and disease-specific manner and translate these findings into meaningful clinical outcomes. In the past few years, as part of our studies to understand signaling pathways and identify novel targets in hormone-dependent cancers, we identified several novel nuclear receptor coregulators that bind a specific RNA coregulator (SRA, Steroid receptor RNA Activator) which is aberrantly expressed in human breast cancer (BCa). These SRA-binding coregulators contain distinct RNA-binding domains from two different structural families, and each modifies SRA-mediated coregulation of multiple nuclear receptor signaling pathways, including estrogen, androgen, glucocorticoid and thyroid. For example, SLIRP is a novel protein that corepresses the estrogen, thyroid and vitamin D nuclear receptor signaling pathways, interacts with multiple other coregulators, including SHARP, SKIP and NCoR, and augments the effects of tamoxifen. Remarkably, the majority of SLIRP resides in the mitochondrion, and it is most highly expressed in the energy-rich tissues (heart and skeletal muscle). Furthermore, SLIRP represses PPARδ signaling suggesting an important role in energy homeostasis and metabolism in heart and skeletal muscle. Taken together, our studies provide insight into the important contribution of SRA-protein interactions to nuclear receptor transcription, support a key physiological role for each of these SRA-binding coregulators in a wide range of nuclear receptor signaling, especially estrogen signaling in BCa and suggest mechanisms by which aberrant expression could modulate anti-estrogen therapies. Furthermore, they illustrate the bifunctional nature of some coregulators, and for SLIRP, suggest key roles in both hormone-dependent cancer and energy homeostasis signaling pathways.

034

INTRACELLULAR STRESS, MITOCHONDRIAL DYSFUNCTION AND DIABETES COMPLICATIONS

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Intracellular stresses, such as oxidative stress and endoplasmic reticulum (ER) stress, are involved in the development of various diseases. In this lecture, our studies about the impacts of ER stress and reactive oxygen species derived from mitochondria (mtROS) on diabetes and its complications will be presented. The ER plays important functions essential to cell survival. Various conditions that interfere with ER function are called ER stress. Severe ER stress leads to apoptosis through induction of ER stress-associated apoptosis factor CHOP. The Akita mouse with a mutation (Cys96Tyr) in the insulin 2 gene develops diabetes with a reduced beta -cell mass. Overexpression of the mutant insulin in MIN6 cells induced CHOP expression and apoptosis. Targeted disruption of the CHOP reduced islet cell apoptosis and delayed the onset of diabetes in Akita mice. In addition, db/db mice displayed an increase of CHOP and other ER stress-related genes suggesting the involvement of ER stress in the progression of diabetes. The mtROS may play primary role in the development of diabetic complications. In MIN6 cells, hyperglycemia increased mtROS production, and the treatment of the beta-cells with H2O2 suppressed the first phase of glucose-induced insulin secretion. On the other hand, in hepatoma Huh7 cells, mtROS decreased tyrosine phosphorylation of IRS-1, an important insulin signal, via activation of ASK-1-JNK pathway. Therefore, mtROS could prevent insulin secretion and action, which may explain glucotoxicity in diabetes. To further study the role of mtROS in diabetic complications, we created a transgenic (eMnSOD-Tg) mouse that overexpresses MnSOD in endothelial cells. Expression of VEGF and fibronectin mRNAs in retinas was observed in STZ-induced diabetic WT mice, which was completely prevented in diabetic eMnSOD-Tg mice. In addition, the increase of 8-OHdG, a marker of oxidative stress was also suppressed in diabetic eMnSOD-Tg mice. In the relative hypoxia-induced in vivo retinopathy model, retinal flat-mount pictures showed typical central avascular areas in WT mice, which were reduced in eMnSOD-Tg mice. Therefore, normalizing hyperglycemia-induced mtROS could prevent diabetic complications in vivo. Our results indicate that intracellular stresses could be novel targets for prevention and treatment of diabetes and its complications.

035

NOVEL APPROACHES FOR THE TREATMENT OF DIABETES - WEARABLE ARTIFICIAL ENDOCRINE PANCREAS (AEP) AND MILD ELECTRIC AND THERMO GENERATOR (MET)

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Diabetic treatment is sometimes difficult in cases of insulin deficient status and of severe insulin resistance. To conquer these, our group has created a wearable artificial endocrine pancreas (AEP) and an instrument, named MET (mild electric current and thermo generator), which reduces insulin resistance in vivo by mild hyperthermia and electric current. AEP is a closed-loop system with glucose sensor, insulin infusion algorithm, and infusion pump to establish strict glycemic control. To establish the ideal insulin delivery route for AEP, we examined the effectiveness of portal and intraperitoneal insulin delivery routes. The closed-loop portal insulin delivery was feasible with regard to both insulin profiles and hepatic glucose handling in vivo. On the other hand, intraperitoneal route is less invasive and ~70% of infused-insulin could flow into portal vein, and achieved better glycemic control when compared with subcutaneous infusion. Therefore, the portal vein may be the most ideal insulin delivery route but intraperitoneal route could also be beneficial for glycemic control by AEP. Hyperthermia is known to reduce insulin resistance, at least in part through expression of HSP72. We recently found that combination of mild hyperthermia and weak electrical current could efficiently induce HSP72 in culture cells. Therefore, an instrument that can induce mild hyperthermia and weak electrical current in vivo, named MET, was created and applied for model mice of insulin resistance. Treatment of the high fat fed mice with MET twice a week for 8 weeks significantly reduced subcutaneous and visceral fat, reduced fasting insulin level, increased adiponectin level and improved glucose tolerance when compared with sham-treated mice. Fatty liver was also dramatically improved. The HSP72 induction was confirmed in tissues of MET treated mice, which paralleled with improved insulin signaling and increased insulin-stimulated GLUT4 translocation. Therefore, MET could be used for the treatment of patients with type 2 diabetes and/or metabolic syndrome.

036

INHALED INSULIN IN DIABETES TREATMENT

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The alveolar membrane is thin, permeable and has a large surface area. Unlike the gastrointestinal tract, it is relatively free of proteases. It therefore represents a suitable site for insulin delivery provided the hormone can be deposited in sufficient local concentration. Various techniques have been developed to make insulin particles of different dryness

and density to optimise its delivery by the inhaled route. Overall, absorption has been found to be reproducible with a rapid onset of action similar to the short acting insulin analogues now commonly in clinical use. However it seems to have a slightly longer duration of action which may stem from a gradual release of some pulmonary deposition of the inhaled insulin. In clinical trials now lasting up to 2-3 years, inhaled insulins have been shown to be non-inferior to subcutaneous insulin in both type 1 and type 2 diabetes and possibly associated with less hypoglycaemia. Its introduction is often associated with a transient cough. There is also a slight deterioration in lung function which is small and generally reversible after cessation of the inhaled insulin treatment. No other significant long term side effects have been demonstrated and one brand of inhaled insulin has already received regulatory approval for marketing in both Europe and U.S.A. Patients treated with inhaled insulin have shown good acceptance to the treatment and a better quality of life. Inhaled insulin is obviously more expensive than conventional products. Whether it can increase willingness to initiate insulin therapy and therefore reduce the morbidity of diabetes in the longer term is an important question yet to be answered.

037

INSULIN DELIVERY BY GENETIC ENGINEERING

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Type I diabetes is caused by the autoimmune destruction of the pancreatic beta cells. The only treatment for the disease is the injection of insulin to regulate blood glucose. Despite the best glucose-monitoring procedures the chronic complications of diabetes still develop: retinopathy, neuropathy, nephropathy and macrovascular complications. The only “cure” for diabetes is the transplantation of donor pancreatic tissue, but this is limited by lack of donors and the fact that patients must be immunosuppressed. Ultimately, other cures may come from xenotransplantation, generation of beta cells from human embryonic stem cells, or gene therapy by the creation of a surrogate beta cell. At the present time, xenotranplantation and stem cell therapy are both fraught with logistic, ethical and legal issues. My laboratory is investigating the use of somatic cell gene therapy as an alternate strategy for reversing diabetes. This strategy is based on the engineering of liver cells to synthesise, store and secrete insulin to glucose and other stimuli, thereby regulating patient blood glucose levels without the need for immunosuppression.

In collaboration with Prof. Tuch at Prince of Wales Hospital we have engineered a liver cell line, Huh7ins that responds in a regulated fashion to a glucose stimulus and corrects diabetes in an animal model. These cells are not destroyed by cytokines of the immune system that precipitate diabetes and this, or a similar cell line could possibly be used clinically following encapsulation and transplantation. An alternative strategy that we are also pursuing is the direct delivery of the insulin gene to the livers of diabetic animals. This procedure has resulted in normalisation of blood glucose levels for 500 days in streptozotocin-diabetic rats, storage of insulin in secretory granules and normal glucose tolerance. These studies give hope that gene therapy may be a treatment for Type I diabetes.

038

TESTOSTERONE DELIVERY - ROUTES OF ADMINISTRATION

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Since the first clinical use of testosterone in 1937 by frequent IM injection there has been a search for more convenient modes of delivery. Low oral bioavailability has led to almost exclusive use of parenteral preparations and the short half-life of testosterone means that it is best delivered by depot preparations.

Despite being available since the 1940's testosterone implants have been curiously neglected as a treatment modality until recent years. A single subcutaneous insertion of four 200 mg pellets maintains adequate testosterone levels in most men for 5 to 6 months. Side effects are infrequent and usually minor (bruising, bleeding, infection) although extrusion of one or more pellets occurs after about 10% of procedures. The major disadvantage of implants is the need for a minor surgical procedure by a trained operator.

Injectable testosterone esters provide reliable and adequate delivery of testosterone. Testosterone enanthate and combinations of esters approximate steady state levels when given weekly but for convenience are usually given second weekly, resulting in supraphysiological levels in the first few days and low levels towards the end of the second week. The recently availability of injectable testosterone undecanoate, which has been used at 12 weekly intervals, is likely to provide a significant advance in injection therapy.

Unlike synthetic 17-alkylated androgens which are potentially hepatotoxic oral testosterone undecanoate is safe. However multiple daily doses are needed and bioavailability is low and erratic. Trandermal preparations offer the same advantages of oral TU with better bioavailability.

The search for a satisfactory transdermal preparation has been hampered by the need for absorption of mg per day. Non-scrotal patches are large and absorption enhancers lead to a high incidence of skin irritation. Testosterone gel is better

tolerated than patches but care needs to be taken to avoid inadvertent transmission to female partners or children. Their short duration of action makes patches and gel particularly suitable for initiation of therapy and in situations where rapid withdrawal of therapy may be required. (e.g. men with treated prostate carcinoma). However they may not provide adequate testosterone delivery in severe androgen deficiency.

041

GENETIC CAUSES OF REPRODUCTIVE FAILURE IN THE MALE

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Infertility affects 1 in 25 Australian men. Of these, 40% present with primary spermatogenic failure (SgF) manifest as a combination of reduced sperm number, motility or structure/function. SgF is a heterogeneous group of disorders in which genetic causes are increasingly being recognized. A greater understanding of such genetic causes is not only essential in the diagnosis and potential treatment of men bearing the condition, but also in understanding the potential implications for children conceived through artificial reproductive technologies. Further, the identification of effective genetic barriers for male fertility represents a valuable tool for the development of novel male gamete based contraceptives.

Chromosomal disorders such as sex chromosome aneuploidies and autosomal translocations are seen in 13.7% of azoospermic and 4.6% of oligospermic men. Y chromosome microdeletions account for 3-5% of men with sperm densities of <5 million/ml, and depending on the region of the Y chromosome deleted present with a spectrum of histopathologies ranging from Sertoli cell only to hypospermatogenesis. More recently work from several groups has suggested that smaller deletions within the same region may or may not critically impair fertility depending upon as yet undefined modifiers on the Y chromosome (the Y haplogroup). Despite the development of many specific gene knockout mouse models with male infertility, the identification of critical single gene lesions in humans has proven to be very difficult. Those that have been identified include the cystic fibrosis transmembrane receptor (CFTR) gene which results in congenital absence of the vas deferens, several genes implicated in primary cilia dyskinesia and a group of genes involved in fibroblast growth factor receptor-1 signaling which are involved in Kallmann's syndrome. To date, however, only a single gene SCP3 has been unequivocally and mechanistically linked to human male SgF in the absence of somatic pathology.

042

ENDOMETRIAL PROPROTEIN CONVERTASE 6: A CRITICAL REGULATOR FOR EMBRYO IMPLANTATION

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Embryo implantation, during which the free-floating blastocyst attaches to and invades the uterus, is vital for mammalian embryo survival and development beyond the blastocyst stage. In women, implantation failure, resulting in embryonic death, is a major cause of early pregnancy loss and female infertility. Implantation failure also limits successful outcome of assisted reproduction (~70% of embryos transferred fail to implant). A better understanding of the molecular mechanisms of implantation is thus critically important in reproductive medicine. Successful implantation requires not only an implantation-competent blastocyst but also an appropriately prepared conducive endometrium. In a broader sense, endometrial preparation for implantation includes (i) differentiation of the endometrium into a receptive state so that at the expected time of implantation, the embryo will be able to attach and adhere to the luminal epithelium, and (ii) conversion of the endometrium into a competent condition so that appropriate tissue responses in the stroma and vasculature, will occur upon the attachment of the embryo, to allow properly controlled trophoblast invasion. We have established that proprotein convertase 5/6 (PC6), a serine protease of the proprotein convertase (PC) family, is a critical endometrial factor for implantation both in mice and primates. PCs control post-translational activation of a range of proteins with important functions (including HIV envelope proteins), and are regarded as “master switch” molecules and potential therapeutic targets (eg. for combating HIV infection). We propose that PC6 regulates endometrial function by activating a cohort of proteins of diverse functions essential for implantation. This presentation will discuss our current understanding of PC6 function in the endometrium and the potential implications of targeting PC6 for fertility control.

043

ENVIRONMENTAL INFLUENCES ON DNA METHYLATION IN EMBRYONIC CELLS: INVESTIGATING MECHANISMS AND PHENOTYPIC CONSEQUENCES.

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The epigenetic reprogramming in DNA methylation that occurs in the preimplantation embryo appears vulnerable to disruption when *in vitro* embryo production technologies are applied and may also be influenced by maternal nutrition *in vivo*. Thus we reasoned that blastocyst-derived, human embryonic stem cells isolated and cultured through a diverse range of protocols in different laboratories (hESC), may also be subject to epigenetic instability and variation (Allegrucci *et al*., 2004. Lancet 364; 206-20), providing a novel model for the human embryo (Allegrucci *et al*., 2005. Reproductive Toxicology 20; 353-367). In order to define the degree of epigenetic variation between independently-derived hESC lines we have employed Restriction Landmark Genome Scanning (RLGS) to examine the genome-wide methylation profiles of gene-rich CpG islands in hESC. Using *NotI/EcoRV/HinfI* digestion, our comparisons of hESC CpG islands to a normal human lymphocyte profile (comprising 2025 fragments for which a genomic *NotI/EcoRV* library was available) have revealed significant epigenetic variation between lines that cannot be accounted for by inherent genetic variability. Studies on the effect of a range of culture conditions revealed epigenetic instability over time in culture, with evidence of stable inheritance of changes occurring at lower passage number. The majority of loci which changed over time within a line were not in common between lines, suggesting that passage–associated changes are stochastic and unpredictable. In contrast, common methylation "hotspots" were identified as changing within the BG01 line when deviations from the standard protocol of culture on mouse embryonic fibroblast feeders with passage by manual dissection were applied. We are currently investigating the phenotypic and therapeutic consequences via examining the derivatives of human embryonic stem cells subjected to different environments expected to influence methyl cycle metabolism. Our data thus far suggest that further optimisation and standardization of hESC culture conditions is urgently required to ensure production of biosafe therapeutic products. Since the environmental factors implicated in altering DNA methylation in hESC cultures are in common with a range of human embryo culture media, hESCs might provide a novel model system to optimise culture conditions for assisted reproduction technologies.

In a complimentary approach to examine phenotypic consequences of methylation-relevant nutrients on programming the embryo *in vivo*, we have developed a sheep model. Maternally-applied methyl group deficiencies in diet during the periconceptual period are being examined for the effects on DNA methylation and subsequent conceptus development. Our rationale for these studies is that identification of key nutrients which can predispose early embryonic cells to programmed epigenetic change might uncover mechanisms pertinent to the developmental origins of adult disease. The latest results of these studies will be presented.

044

A MITOCHONDRIAL COMPONENT TO DEVELOPMENTAL PROGRAMMING

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Adult physiology is not simply dependent on the sequence of the nuclear genes we inherit. There is an increasing appreciation that very early environmental factors can determine development and adult phenotype [1]. Recently, in experimental models it has been clearly demonstrated that environmental stress during pregnancy, particularly during the peri-conceptual period is a determinant of adult disease [2]. Although these findings substantiate the concept of developmental programming – a process by which very early stress is proposed to have detrimental effects on offspring health. The molecular mechanisms of this phenomenon remain unclear. However, epigenetic changes to nuclear DNA (nDNA) have been implicated in this process [3].

We have evidence that programmed deficits in mitochondrial function contribute to adult disorders in several diverse models of developmental programming. Mitochondria require both nDNA and mitochondrial DNA encoded transcripts to function [4]. Therefore persistent changes to either genome could cause abnormal mitochondria. Although we find that different maternal nutrient stresses result in similar deficits in mitochondrial function in adult offspring, namely abnormalities in the activity of mitochondrial electron transport complex iii, we have recently found that this deficit can arise from the environmental disruption of one of a number of distinct cellular processes. Oocytes and preimplantation embryos are particularly prone to environmentally induced changes to mitochondria whose effects can be mimicked in vitro and persist after the initial period of stress [5]. The cause and consequences of these changes will be discussed. Our work has particular relevance to assisted conception procedures and to embryo based stem cell derivation.

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045

METHYLCYTOSINE DEAMINATION BY DNA DEAMINASES AND EXPRESSION IN REPROGRAMMING TISSUES.

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Highly differentiated and specialised gametes undergo epigenetic remodeling essential to reconstitute the diploid nucleus of the zygote and restore totipotency. The remodelling involves DNA methylation and covalent modifications of core histones.

DNA methylation is important for epigenetic regulation of the genome. Through normal development it contributes to parental imprinting, X chromosome inactivation, silencing of transposable elements, and tissue-specific gene expression. It is also implicated in cancer and ageing. DNA methylation reprogramming occurs after fertilisation during early mammalian development and during germ-cell development. The loss of methylation without replication seen in these cases suggests an enzyme-catalysed reaction.

The loss of methylation from cytosine may occur directly but is energetically unfavourable. More likely is the deamination of methylcytosine to thymine followed by repair of the mismatch replacing methylcytosine with cytosine. The deamination of methylcytosine can also lead to mutations. With the discovery of cytosine deaminases and the otherwise paucity of methylcytosine directed activities, we are interested in determining whether these deaminases possessed any methylcytosine deaminase activity. We find that Aid and Apobec1 have robust deaminase activities against methylcytosine measured in both an *in vitro* assay and an *E. coli* based bioassay. These two deaminases are located in a cluster with other genes expressed in pluripotent tissues, and we find expression of these deaminases in oocytes, primordial germ cells and other pluripotent tissues. We are investigating the role of these deaminases in mice.

DNA demethylation is part of the epigenetic reprogramming that takes place in the early embryo and the establishment of the germ line. Understanding epigenetic events during these key phases of development will contribute to our knowledge of a process that is required for normal development and reproduction, and may prove useful in attempts to treat cancer, defer ageing, and reprogram somatic cells through nuclear transfer.

ORALS - ESA submissions

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EVIDENCE FOR A NOVEL MECHANISM FOR HUMAN MYOMETRIAL ACTIVATION

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Background: It has been recently reported that in vascular smooth muscle post-translational modifications (PTM) to small heat shock proteins (HSP20 and HSP27) modulate their interaction with the actin cytoskeleton and thus contractility. The mechanisms regulating human myometrial contractility are unknown but may also involve PTM of regulatory proteins. We sought to identify proteins in human myometrium that undergo PTM with labour using 2-Dimensional Difference In Gel Electrophoresis (2D-DIGE).

Hypothesis: Labour is associated with PTM of regulatory myometrial proteins.

Aim: To identify myometrial proteins that undergo PTM with labour.

Methods: Myometrial biopsies collected during elective Caesarean Section (non-labouring; NL) or following spontaneous labour (L) were extracted and analysed quantitatively using 2D-DIGE (n=6 for both groups). Proteins significantly modified with labour onset (>1.5 fold) were sequenced using MALDI-ToF . Western immunoblotting on NL (n=14) and L (n=11) myometria was performed using antibodies to HSP20, HSP27 and its serine phospho-isoforms (pHSP27-s15, s78 and s82). Immunofluorescence analysis was performed using antibodies to HSP20, HSP27 and smooth muscle α-actin.

Results: 2D-DIGE identified 12 proteins that were significantly altered with labour. α-B-crystallin , a small HSP, showed one of the most profound changes (3.3-fold decrease) with labour onset. α-B-crystallin is known to regulate HSP20 and HSP27 and subsequently their ability to modulate actin-myosin interaction in vascular smooth muscle. Immunoblotting analysis revealed the presence of both HSP20 and 27 in myometrium and a significant increase in the ratio of total HSP27:HSP20 with labour. pHSP27-s15 was increased 3-fold in L myometrium ($P<0.05$). In contrast pHSP27-s82 was decreased 6.5-fold ($P<0.01$). Immunofluorescence revealed HSP27 was co-incident with HSP20 in the perinuclear region of NL myometrium (n=6) while in labouring myometrium (n=6) it was associated with the cytoskeleton.

Conclusion: This is the first study to demonstrate the presence of small HSPs in human myometrium. As they change with the onset of labour and have a known role in regulating contractility in vascular smooth muscle it is likely that they are key regulators of myometrial contractility and may provide a rational target for the development of novel therapeutics for the treatment of preterm labour.

102

THE CLASSICAL ANDROGEN RECEPTOR PATHWAY IN SERTOLI CELLS IS VITAL FOR COMPLETE SPERMATOGENESIS

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Androgen actions are essential for spermatogenesis, yet male germ cells do not express androgen receptor (AR), highlighting the key role for AR in the somatic cells of the testis. Contribution of AR activity in different somatic cells to the overall testicular androgen response remains unclear. We have used a Cre-loxP strategy to selectively study Sertoli cell (SC) AR actions. SC-specific transgenic (Tg) Cre was combined with a floxed-AR gene (*1*) for Cre-mediated inframe excision of exon 3, encoding a zinc finger essential for DNA-binding, designed to produce mutated *AR* $^{\Delta ex3}$ which is unable to bind DNA and directly regulate gene transcription. Two unique SC-specific *AR* $^{\Delta ex3}$ (*SC* $^{AR\Delta ex3}$) models were established: i) *AMH*.SC$^{AR\Delta ex3}$ mice from Tg *AMH* promoter-driven Cre (*2*) X floxed-AR mice, and ii) *ABP*.SC$^{AR\Delta ex3}$ mice from Tg *Abp.Cre* mice X floxed-AR mice. The rat *ABP* promoter directs Sertoli-specific Cre based on our past use (*3*) and verified by *ROSA* reporter mice, although fetal *AMH.Cre* expression (15 dpc, (*2*)) predicts slightly earlier Sertoli AR loss in *AMH*.SC$^{AR\Delta ex3}$ mice compared to later (dpc17) *ABP.Cre* Cre expression driving Sertoli AR loss in *ABP*.SC$^{AR\Delta ex3}$ mice.

Adult *AMH*.SC$^{AR\Delta ex3}$ and *ABP*.SC$^{AR\Delta ex3}$ males exhibit smaller ($p<0.001$) testes (29.8 ± 1.1 and 42.9 ± 2.6 mg respectively) compared to age-matched controls (114.23 ± 1.85 mg). Immunohistochemistry revealed SC *nuclear* AR expression was retained in these smaller *SC* $^{AR\Delta ex3}$ testes. *AMH*.SC$^{AR\Delta ex3}$ testes showed predominant meiotic arrest, compared to more postmeiotic development in *ABP*.SC$^{AR\Delta ex3}$ testes. Both SC$^{AR\Delta ex3}$ mouse models displayed Leydig cell hypertrophy, indicating loss of paracrine AR-mediated regulation. However, both SC$^{AR\Delta ex3}$ models had normal serum T levels and seminal vesicle weights, demonstrating that these models provide a unique opportunity for selective investigation of Sertoli AR function without confounding secondary changes to androgen production. Both *SC* $^{AR\Delta ex3}$

models provide compelling evidence for a pivotal role of Sertoli *genomic* AR function for inducing the completion of meiotic-postmeiotic germ cell development.

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103

EVIDENCE THAT PREMATURE INFERTILITY IN TRANSGENIC FSH FEMALE MICE IS DUE TO AGE-RELATED CHANGES IN EMBRYO SURVIVAL: AGEING OOCYTE OR UTERUS?

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Female fertility declines several years before menopause, coinciding with a 'monotrophic' rise in circulating FSH (unchanged LH), considered one of the first signs of reproductive ageing. As more women in developed countries delay childbearing it is increasingly important to understand the underlying mechanisms of age-related reduced fecundity. Despite important clinical implications, the consequences of rising FSH on ovarian function is little studied, largely due to lack of specific *in vivo* models. We created transgenic (Tg) mice with rising serum FSH and unchanged LH that exhibit larger littersize in young females (<150 do) followed by premature infertility in 150-280 do females compared to nonTg controls. To explain these effects we proposed TgFSH accelerates follicle growth and depletion of follicle reserve. Initial analysis (n =3/group) of preantral development during reduced fertility/infertility revealed little overall change, with slightly reduced small preantral follicle numbers (66.0±1.5 vs 79.0±2.3, $P<0.01$) and increased preovulatory follicle numbers (5.7±1.2 vs 3.7±0.3, $P=0.184$) in 180 do TgFSH females relative to nonTg controls insufficient to explain the infertility. However, corpora lutea counts were increased 5-fold (35.3±2.7 vs 7.0±1.5, $p<0.001$) and 2.8-fold (20.0±4.4 vs 7.0±1.2, $p<0.05$) in 85 do (hyperfertile) and 180 do TgFSH ovaries, respectively, compared to nonTg controls. Thus, TgFSH females maintain follicle development and increased ovulation despite declining fertility. Furthermore, uterine examination revealed increased embryo implantation rates in 85 do (14.2±2.0 vs 8.9±0.6, $p<0.05$) and 180 do (16.1±2.4 vs 7.5±0.6, $p<0.05$) TgFSH compared to nonTg females. Initial findings of increased embryo resorption in 180 do TgFSH compared to nonTg females suggests the FSH-induced age-related decline in fertility is associated with a failure in embryo development or uterine support. We propose this TgFSH paradigm will be invaluable in determining FSH induced age-related changes to female fertility via changes in ovarian and/or uterine function relating to oocyte and embryo quality or post-implantation survival.

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REDUCED 11β HYDROXYSTEROID DEHYDROGENASE-1 IN SKELETAL MUSCLE IN TYPE 2 DIABETES: INDUCTION BY DEXAMETHASONE

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11β HSD-1 converts cortisone biologically active to cortisol. H6PDH generates NADPH, promoting oxoreductase activity in intact cells. Studies have examined 11β HSD-1 in liver and adipose tissue, but little data exist regarding expression in human skeletal muscle. We aimed to 1. determine if there are biologically relevant amounts of 11β HSD-1 and H6PDH in fresh skeletal muscle 2. compare basal 11β HSD-1 activity in skeletal muscle of diabetic subjects and healthy controls and the response to dexamethasone and 3. demonstrate protein localisation and fibre type distribution of 11β HSD-1. 12 subjects with Type 2 diabetes (not on insulin) and 12 controls underwent muscle biopsy of vastus lateralis at baseline and after 4 days of treatment with 4mg dexamethasone/day. 11β HSD-1 and H6PDH mRNA expression were assessed by quantitative real time RT-PCR, 11β HSD-1 activity was estimated by conversion of ^{3}H cortisone to cortisol, and 11β HSD-1 protein localisation and fibre type specificity by immunohistochemistry. Baseline 11β HSD-1 activity was reduced in diabetic subjects; percent conversion of ^{3}H cortisone to cortisol 11.4 ± 2.5% (diabetic) vs 18.5 ± 2.2% (control) ($P<0.05$). After dexamethasone, 11 b HSD-1 activity increased significantly only in the diabetics ($P<0.05$). Basal 11β HSD-1 mRNA expression was similar, and increased significantly following dexamethasone in both groups ($P<0.001$), but there was no change in H6PDH mRNA expression. H6PDH mRNA was similar in diabetic and control subjects and correlated with 11 b HSD-1 mRNA ($R=0.65$, $P=0.001$) but was approximately 35 fold more abundant. 11β HSD-1 protein staining was present in all specimens, with similar distribution amongst fast and slow twitch fibres. Our findings are consistent with a reduction in 11 b HSD-1 activity being a protective mechanism in unfavourable metabolic states. High levels of H6PDH should ensure that 11β HSD-1 oxoreductase activity is predominant *in vivo*. 11β HSD-1 location within skeletal muscle is not fibre type specific.

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POSTNATAL GROWTH IS IMPROVED BY CROSS-FOSTERING A PUP BORN SMALL ONTO A MOTHER WITH NORMAL LACTATION BY ALTERING ALVEOLAR AREA, MILK PRODUCTION AND MILK PROTEIN GENE EXPRESSION

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Being born small and subsequent accelerated postnatal growth have been implicated as contributing to the increased risk of developing adult diseases. We have shown that placental restriction in the rat impairs fetal growth, mammary function, pup milk intake and postnatal growth. Our aim was to determine whether a pup born small could improve its' growth by suckling on a mother with normal mammary function through effects on alveoli area, milk production and milk protein gene expression. Bilateral uterine vessel ligation (Restriction, R) or Sham control surgery (Control, C) was performed on day 18 of gestation in Wistar Kyoto rats. Control, Reduced (reduced litter size of Control to 5, to match Restricted) and Restricted pups were cross-fostered onto a Control or Restricted mother on postnatal day 1. Pup weight and milk intake were measured. Maternal mammary glands were analysed histologically for alveoli area and for mRNA expression of milk protein genes. On day 6 after birth, R-on-R pups were smaller and had a lower milk intake than all other groups and had a lower alveoli area than C-on-C mothers ($p<0.05$). By day 6, R-on-C pups increased their milk intake and growth to C-on-C. Fostering a C pup on a R mother rescued mammary morphology by increasing alveoli area. Conversely, fostering a R pup onto a C mother, reduced alveoli area to that of R-on-R. Milk protein gene expression was higher in R mothers during pregnancy. This study demonstrates that placental restriction reduces pup body weight which can be increased by suckling on a mother with normal lactation (R-on-C) despite the low alveoli area. The premature initiation of lactation following placental restriction during pregnancy may have consequences for milk protein expression and hence postnatal growth. The altered lactation environment not only has consequences for postnatal growth but also adult disease development.

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REGULATION OF VOLTAGE-GATED CA^{2+} CURRENTS OF RAT SOMATOTROPES THROUGH SUBTYPES OF SOMATOSTATIN RECEPTORS

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The secretion of growth hormone (GH) is mainly regulated by hypothalamic GH-releasing hormone (GHRH) and somatostatin (SRIF). Five subtypes (SSTR1-5) of SSTRs have been identified. SRIF increases voltage-gated K^+ currents through activation of SSTR2 and SSTR4 (1) through Gi3 proteins in rat somatotropes (2). In contrast, SRIF decreases voltage-gated Ca^{2+} currents through Go2 proteins (3) via unidentified SSTR subtypes. In this study, we tested for a link between subtypes of SSTRs and Ca^{2+} currents in the GH 3 rat somatotrope cells using specific SSTR agonists, L-797,591 (SSTR1), L-797,976 (SSTR2), L-797,778 (SSTR3), L-803,087 (SSTR4), L-817,818 (SSTR5>SSTR1). ALL five SSTR mRNAs are detected in GH 3 cells by RT-PCR, with dominant expression of the SSTR2 gene. Nystatin-perforated whole cell patch-clamp configuration was employed to record voltage-gated Ca^{2+} currents with a holding potential of -80mV in the presence of K^+ and Na^+ channel blockers. Ca^{2+} currents were composed of L, N, P/Q and T types and SRIF reduced L, N but not T, P/Q currents in GH 3 cells. Activation of SSTR2 with 10^{-7} M and 10^{-8} M of L-797,976 decreased voltage-gated Ca^{2+} currents and abolished any further decrease by SRIF. SSTR1, SSTR3, SSTR4 and SSTR5 agonists at 10^{-7} M did not modify voltage-gated Ca^{2+} current and did not affect the Ca^{2+} current response to SRIF. These results indicate that SSTR2 is mainly involved in regulating voltage-gated Ca^{2+} currents by SRIF. Functional relationship of SSTR subtypes and SRIF-induced inhibition of GH secretion is currently under investigation.

Key Words: somatostatin, Ca^{2+} current

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A POSTNATAL DIET RICH IN OMEGA-3 FATTY ACIDS ATTENUATES GLUCOCORTICOID-PROGRAMMED HYPERINSULINEMIA BUT DOES NOT ALTER ABERRANT PROGRAMMED GENE EXPRESSION IN SKELETAL MUSCLE.

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We recently demonstrated that postnatal ingestion of omega-3 fatty acids can attenuate glucocorticoid-induced programming of hypertension and hyperleptinemia in the rat (Wyrwoll *et al.*, *Endocrinology* 2006 147 :599-606). The

present study determined programmed changes in plasma insulin, serum glucose, hepatic GR and PEPCK expression, and skeletal muscle (gastrocnemius) GLUT4, PPARδ and UCP3 expression. We also determined whether any programmed changes were altered by a postnatal diet rich in omega-3 fatty acids. Dexamethasone was administered to pregnant rats (0.75 µg/ml in drinking water) from day 13 to term. The offspring of treated and control mothers were cross-fostered to mothers on either a standard or high omega-3 diet, and remained on these diets post-weaning. In six month old rats, insulin was measured by radioimmunoassay, glucose by a hexokinase assay and mRNA expression of GR, PEPCK, GLUT4, PPARδ and UCP3 were assessed by real time quantitative RT-PCR. Hyperinsulinemia was evident in offspring exposed to dexamethasone *in utero* and raised on a standard diet, but this effect was completely blocked by a high omega-3 diet from birth. Serum glucose was unaffected by either prenatal treatment or postnatal diet. Despite elevation of hepatic GR in all dexamethasone-exposed offspring, PEPCK expression was unaffected. In skeletal muscle, GLUT4 expression was elevated by up to 20-fold in dexamethasone-exposed offspring but this effect was not altered by postnatal diet. Fetal glucocorticoid excess also increased skeletal PPARδ expression and decreased UCP3 expression, but again neither change was affected by diet. These results demonstrate for the first time that a postnatal diet high in omega-3 fatty acids can attenuate glucocorticoid-induced programmed hyperinsulinemia. Additionally, skeletal muscle in the programmed offspring exhibits aberrant expression of genes involved in glycaemic control, but this effect is not altered by postnatal diet.

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A LACK OF PERIPHERAL TISSUE RHYTHMICITY ALTERS METABOLIC HOMEOSTASIS IN MICE

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Shiftworkers have a higher prevalence of diabetes and cardiovascular disease compared to day workers suggesting that the abnormal rhythmicity provoked by shiftwork schedules may be a causal factor in the metabolic syndrome.

Melatonin proficient *Clock* $^{\Delta 19}$ +MEL mutant mice with normal central, but disrupted peripheral organ rhythmicity [1] were therefore used to address the role of circadian rhythmicity in metabolic homeostasis. Blood, liver, adipose tissue (epigonadal) and muscle were collected 4 hourly over 24 hours. Intraperitoneal glucose tolerance tests were also performed.

Clock $^{\Delta 19}$ +MEL mice had lower plasma glucose during the light period in males (but not females), lower plasma free fatty acids, lower plasma insulin (males), increased plasma adiponectin and impaired glucose tolerance than wild type mice. Expression of *Gck*, *Pfk* and *Pepck* mRNA in liver was rhythmic (highest around the light-dark transition) in wild type mice, but arrhythmic and lower in Clock $^{\Delta 19}$ +MEL mice suggesting impairment of both glycolysis and gluconeogenesis. In adipose tissue, mRNA expression of the clock genes *Bmal1* and *per2* in wild type mice showed a high amplitude rhythm, but adiponectin and leptin mRNA expression did not change across the day and was similar in wild type and mutant mice. In skeletal muscle, *Glut4* mRNA was reduced in Clock $^{\Delta 19}$ +MEL mutants, while the patterns of *Ppar a* and *cpt1* mRNA expression were unaltered.

These studies provide compelling evidence for a role of circadian rhythmicity in the glucose/insulin and adipoinsular axes. Disruption of tissue rhythmicity in shiftwork, in conjunction with poor diet and sleep loss may contribute to metabolic syndrome.

(1) DJ Kennaway, JA Owens, A Voultsios and TJ Varcoe Functional central rhythmicity and light entrainment, but not liver and muscle rhythmicity are Clock independent. Am J Physiol (in press 2006)

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FREE FAT ACIDS (FFAS) STIMULATE CA^{2+} RELEASE FROM IP_3-SENSITIVE CA^{2+} STORAGE SITES AND REDUCE VOLTAGE-GATED CA^{2+} CURRENTS IN PRIMARY CULTURED RAT PANCREATIC β-CELLS

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FFAs in a short term stimulate insulin secretion from pancreatic β-cells but the mechanism underlying such stimulation is unclear. GPR40 is proposed to be specific receptor for FFAs and abundantly expressed in β-cells. Linoleic acid is a high affinity ligand to GPR40 and reported to decrease voltage-gated K^+ currents in rat β-cells[1]. The aim of this study was to clarify the direct effect of linoleic acid on voltage-gated Ca^{2+} currents in rat β-cells. Nystatin-perforated whole-cell recording was employed to record voltage-gated Ca^{2+} currents and confocal microscope was used to measure $[Ca^{2+}]i$ change indicated by Fluo-3 fluorescent intensity. Using 5 mM Ca^{2+} bath solution containing TEA (40 mM, K^+ channel blocker) and TTX (1 µM, Na^+ channel blocker) and pipette solution containing Cs^+ (140 mM, to replace K^+), two types of voltage-gated Ca^{2+} currents were isolated as the long-lasting (L) and transient (T) currents. Major component of the total Ca^{2+} currents was the L current. Linoleic acid significantly and reversibly decreased the

amplitude of both L and T currents, while methyl-linoleate, negative control reagent with no binding affinity to GPR40, did not alter the amplitude of currents. The linoleic acid-induced reduction in Ca^{2+} currents was sustained when cells were pre-incubated with PKA inhibitor H89 (1 μM) or PKC inhibitor chelerythrine (5 μM) but was completely abolished by 30 min pre-incubation with $InsP_3$-sensitive Ca^{2+} store depleting reagent thapsigargin (1 μM) or 10 min pre-incubation with $InsP_3$-receptor blocker 2-APB (5 μM). Under confocal microscope, linoleic acid induced an immediate increase in $[Ca^{2+}]i$ through $InsP_3$-sensitive Ca^{2+} release. We conclude that linoleic acid acts on GPR40 and evokes Ca^{2+} release from $InsP_3$-sensitive Ca^{2+} pool, which in turn increases $[Ca^{2+}]i$ levels leading to a reduction of voltage-gated Ca^{2+} currents in rat β-cells. Supported by NHMRC and Eli Lilly Australia.

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REGIONAL DIFFERENCES IN ENDOTHELIAL DYSFUNCTION IN DIABETES MELLITUS

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Endothelial dysfunction is an inevitable vascular complication of diabetes. Nitric oxide (NO) and endothelium-derived hyperpolarizing factor (EDHF) contribute to vasodilation, the latter being particularly important in small blood vessels. Both NO and EDHF are impaired in diabetes. Here we examined the role of tissue oxidative stress and the ion channels mediating EDHF in endothelial dysfunction in mesenteric and hindlimb beds in the streptozotocin (STZ)-induced diabetic rat model.

Eight weeks after diabetes induction, mesenteric and femoral arteries were isolated for *in vitro* measurements: (1) membrane potential and tension, (2) superoxide production using lucigenin-enhanced chemiluminescence. *In vivo*, blood flow was recorded in mesenteric and hindlimb beds using a transit-time ultrasound flow probe in anaesthetized rats. In all experiments the endothelium was stimulated using acetylcholine (ACh) and NO synthase and small- (SK_{Ca}) and intermediate- (IK_{Ca}) conductance, calcium-activated K^+ channels mediating EDHF were blocked using selective blockers as required.

EDHF-mediated hyperpolarization and relaxation was halved in mesenteric arteries of diabetic rats, and the contributions of both SK_{Ca} and IK_{Ca} to EDHF-mediated responses were equally reduced. *In vivo* blood flow recordings demonstrated impaired contribution of EDHF to ACh-mediated vasodilation in the mesenteric bed in diabetes, and the contribution of NO was also reduced, reflecting reduced bioavailability. In the hindlimb bed, the contribution of NO to ACh-evoked vasodilation was upregulated while that of EDHF was reduced, with an overall preservation of perfusion. Superoxide production was significantly upregulated in mesenteric but unaltered in femoral arteries in diabetes.

In conclusion, here we demonstrate the existence of regional differences in endothelial vasodilator dysfunction in diabetes. We also show reduced activities of both SK_{Ca} and IK_{Ca} channels, and this accounts for the impaired EDHF response in small resistance vessels. Our *in vivo* results suggest that regional variability in oxidative stress may contribute to differences in NO responses.

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PREFERENCE BETWEEN EXERCISE AND EATING IS INFLUENCED BY PRENATAL NUTRITION AND OBESITY DEVELOPMENT IS PREVENTED BY PROVIDING AN OPPORTUNITY TO EXERCISE.

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The rapid rise in obesity suggests a central role for environmental factors that promote energy storage. Previous studies in rats show that offspring of mothers undernourished during pregnancy develop obesity and hyperinsulinaemia under standard laboratory conditions. We have investigated the long-term effects of prenatal nutrition on preference of running versus eating and monitored the consequences of these choices on obesity development and endocrine parameters.

Virgin Wistar rats were time-mated and assigned to receive chow either *ad-libitum* (AD) or at 30% of *ad-libitum* intake (UN) throughout pregnancy. Male offspring were divided into four groups: AD Non-Exercised (AD-N), AD Exercised (AD-E), UN Non-Exercised (UN-N), UN Exercised (UN-E). From 60 days of age rats were placed daily in an operate chamber for one hour and were given the choice between running in an exercise wheel and pressing a lever for food. Non-exercised groups could only press a lever for food. The number of lever presses and wheel quarter turns completed in every session were recorded for 200 days. UN-E offspring showed a consistently greater preference for running over lever-pressing for food (AD-E vs UN-E $P<0.05$) throughout the entire study. Body composition was quantified using Dual X-ray Absorptiometry (DEXA) at 45, 100, 150 and 250 days of age and blood samples were taken from the tail for

endocrine analysis. While UN-N offspring developed obesity and hyperinsulinaemia at 150 days, obesity development and hyperinsulinaemia were prevented in UN-E by offering the opportunity to exercise daily (% body fat AD-N 24.2±1.6, UN-N 29.7±2.2, AD-Ex 23.6±1.8, UN-Ex 25.2±1.7, AD-N vs UN-N P<0.05, fasting insulin(μg/L)AD-Non 1.0±0.2, UN-Non 1.8±0.4, AD-Ex 0.9±.4, UN-Ex 1.4±.0.2 AD-N vs UN-N P<0.05).

Offspring from dams undernourished during pregnancy showed a greater preference for running over lever-pressing for food. Providing these offspring with the opportunity to exercise prevented the development of obesity and metabolic abnormalities.

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THE EFFECT OF ESTROGEN ON TRIGLYCERIDE HOMEOSTASIS

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The Aromatase Knockout (ArKO) mouse is estrogen-deficient due to the deletion of exon IX of the aromatase (*Cyp19*) genes. Male and female ArKO mice both develop phenotypes of metabolic syndrome, including insulin resistance, obesity and dyslipidemia (1). However only male ArKO mice develop hepatic steatosis (fatty liver), which is reversible upon estrogen replacement (2). The fatty liver phenotype coincides with the occurrence of apoptosis in the hypothalamic arcuate nucleus (regulates energy homeostasis), which is also male ArKO-specific (3). This study aims to investigate the role of estrogen receptors (ER) in the regulation of triglyceride (TG) homeostasis. Nine-month old WT and ArKO male mice received daily subcutaneous administration of either ERα- or ERβ-specific agonist (Schering) for 6 weeks. Treatment with ERα-agonist dramatically alleviated hepatic steatosis in the male ArKO mice (Fig. 1) , with a significant reduction in liver TG content (p <0.05) , whilst treatment with ERβ-specific agonist resulted in only a modest reduction. Real-time PCR data confirmed that ERα but not ERβ was expressed in the adult mouse liver, and there was no difference in levels of expression between male and female, WT or ArKO mice. Treatment with ERα-agonist also significantly reduced gonadal and visceral adipose tissue weights (p<0.05), as well as total serum TG content below that of WT (p<0.05). Such reductions by ERβ-agonist treatment were insignificant. These observations suggest that estrogen predominantly acts via ERα to regulate TG homeostasis, most likely at a local (liver and adipose tissues) and/or central level.

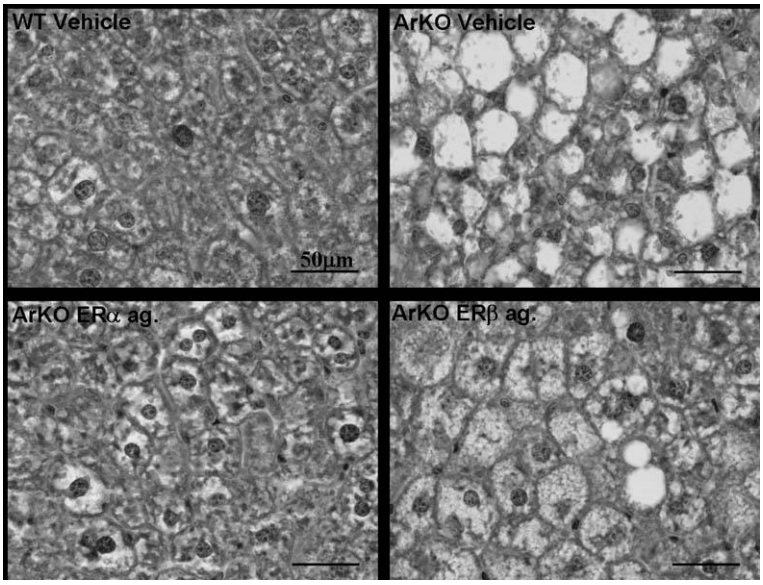

Figure 1. Liver histology of nine month-old male mice treated for 6 weeks, a) WT vehicle control, b) ArKO vehicle control, with lipid droplets c) ArKO with ERα agonist and d) ArKO with ERβ agonist.

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CIRCULATING PORCINE GHRELIN CONCENTRATIONS ARE RESPONSIVE TO ENERGY METABOLITES AND NOT INSULIN

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Ghrelin is a recently discovered orexigenic peptide of which its endogenous initiation is not fully understood. Previously, we have shown that the neonatal pig displays two distinct metabolic states, with carbohydrate or lipid used as the primary energy substrate at different points of development.

This study seeks to determine the responsiveness of ghrelin to changes in metabolic status induced by acute fasting.

Table 1. Neonatal piglets either remained (N=13) on sows or separated (N=19) between 12h and 24h post-partum and sampled following euthanasia at 24h and 108h post-partum.

Piglet treatment	Remained on sow		Separated from sow	
Sampling time post-partum	24h	108h	24h	108h
Plasma Ghrelin (pg/mL)	209±76.7[a]	44±7.5[b]	390±169[a]	39±5.6[b]
NEFA's (mEq/mL)	0.20±0.03[a]	0.82±0.45[b]	0.14±0.01[b]	0.58±0.23[b]
Glucose (mMol/L)	5.73±0.52[a]	9.26.0±0.92[b]	3.74±0.33[b]	9.27±0.77[b]
Insulin (ng/mL)	0.98±0.33[a]	0.87±0.29[a]	0.01±0.02[b]	1.81±0.47[a]
Pancreatic Ghrelin (pg/mL/g)	457±93	632±198	372±93	572±110

[ab] Mean ± SEM superscripts differ significantly ($P \leq 0.05$)

Feed deprivation increased plasma ghrelin at 24h compared to fed animals. Fasting significantly reduced ($P<0.05$) plasma insulin, glucose and NEFA concentrations.

Circulating ghrelin levels in piglets remaining on the sows at 24h were significantly higher ($P<0.05$) than those at 108h. At 108h NEFA and glucose concentrations were also significantly higher ($P<0.05$) suggesting a negative correlation between energy substrate and circulating ghrelin. Ghrelin concentrations in piglets remaining on sows decreased significantly ($P<0.05$) over time with no corresponding change in insulin. In addition there was no alteration in pancreatic tissue ghrelin synthesis in response to insulin.

Therefore the differences in circulating ghrelin in fed animals between 24h and 108h suggests that ghrelin is responsive to mobilization of energy substrate rather than to an alteration in insulin secretion.

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INCREASED FAT MASS IN ANDROGEN RECEPTOR KNOCKOUT MICE DEMONSTRATES A ROLE FOR ANDROGENS IN REGULATION OF FAT MASS AND METABOLISM IN MALES

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Low androgen levels are associated with increased risk of central obesity in males, which is a risk factor for type 2 diabetes and cardiovascular disease. We are using global androgen receptor (AR) knockout (ARKO) mice to study AR-mediated actions of androgens in regulating fat mass and metabolism in males. Exon 3 of the AR gene is deleted in ARKO mice, generating a mutant receptor lacking DNA-binding and transactivation activity. Body mass of mice was measured at 9-30 weeks of age, and fat pad mass at 9 and 12 weeks. At 9 weeks, body mass of ARKO males is intermediate between that of wildtype (WT) males and females, and this difference is maintained at 12-30 weeks. Despite the decreased body mass, subcutaneous fat mass is significantly increased in ARKO males at 9 weeks (99.7±3.9 vs 129.1±9.6 mg, WT versus ARKO male (mean±SEM), $n \geq 15$/group, $p=0.006$, Student's t-test) and 12 weeks (142.7±8.0 vs 242.0±27.4 mg, WT versus ARKO male, $n \geq 7$/group, $p=0.001$). Infrarenal fat mass is also significantly increased in ARKO males at 12 weeks (42.5±4.7 vs 65.2±10.2 mg, WT versus ARKO male, $n \geq 7$/group, $p=0.032$). Infrarenal fat mass is >2 SD greater than the WT mean in 5/15 ARKO males at 9 weeks and 4/7 ARKO males at 12 weeks, suggesting the phenotype is incompletely penetrant. Microarray analysis of muscle mRNA from ARKO and WT males has identified ~120 androgen-responsive genes. Gene ontology mining revealed biological processes over-represented in this group, including regulation of insulin signalling, glucose/lipid transport and cholesterol metabolism, suggesting androgen action in muscle contributes to the metabolic phenotype. Quantitative real-time PCR is being used to confirm these results. These data demonstrate that androgens acting via the AR contribute to regulation of fat mass in males, and we are currently investigating the mechanisms underlying this action.

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INHIBIN α SUBUNIT WITH AN A257T MUTATION IS ASSOCIATED WITH PREMATURE OVARIAN FAILURE: IS THIS INHIBIN FORM BIOACTIVE?

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A naturally occurring missense mutation (769G>A) resulting in a non-conservative change of alanine to threonine in the inhibin a subunit (*INHA*, A257T) has been shown to be associated with POF. The aim of this study was to demonstrate that this mutation impairs inhibin bioactivity in several *in vitr*o systems. Two transfection reporter systems were used to assess inhibin antagonism of activin A-stimulated FSHβ and GnRHR promoter activity in pituitary gonadotroph (LβT2) and ovarian (COV434) cells. Overexpression of the mutant inhibin showed significantly less suppression of FSHβ and GnRHR transcriptional activity in LβT2 cells compared to wild type ($P = 0.02$, $P = 0.0001$ resp, n = 6). In COV434 cells, the mutant inhibin was less bioactive than wild type in suppressing GnRHR promoter activity ($P<0.0001$, n=6). This decrease in bioactivity was not attributable to reduced inhibin α-β subunit dimerisation based on similar inhibin levels measured by immunoassay in wild type and mutant inhibin cultures. These findings were confirmed by assessing the *in vitro* activity of purified wild type and mutant inhibin A and B forms to suppress FSH secretion in rat pituitary cells. When expressed as a ratio of *in vitro* bioactivity to inhibin immunoactivity, the bioactivity of mutant inhibin B was also reduced. It is concluded that the mutated inhibin α subunit, in dimeric α-β subunit form, is less active than wild type forms in these *in vitro* systems and provides a basis for the higher incidence of POF in women with this mutation. This reduction in inhibin bioactivity could result in an accelerated loss of follicles leading to POF. The current study highlights the importance of inhibin in the female reproductive system and provides further insights into the genetic aetiology of POF.

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NEUTROPHIL DEPLETION RETARDS ENDOMETRIAL REPAIR

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Inflammatory cells are highly abundant within the endometrium prior to and during menstruation. We hypothesise that these contribute functionally to menstruation and/or endometrial repair. As menstruation only occurs in women and few other species, lack of suitable animal models make functional studies difficult. We developed a unique mouse model of endometrial breakdown and repair, in which decidualisation is artificially induced, and progesterone support withdrawn; endometrial tissue progressively breaks down by 24h after progesterone withdrawal and by 48h has usually undergone complete repair, morphologically resembling human endometrium at menstruation. In this study, the presence and localisation of markers for key inflammatory cells were examined in our model, and the functional contribution of neutrophils determined.

Immunohistochemistry revealed neutrophils as the most abundant leukocyte. They were rare in decidual tissue, elevated during breakdown and most abundant during early repair. To assess their functional contribution to these processes, the antibody RB6-8C5 was administered to deplete neutrophils. Significant depletion was confirmed both within the circulation (control: neutrophils 22.3±2.4% of total leukocytes, RB6-8C5 treated: 3.8±0.3%, $p<0.0001$) and tissue.

To ascertain whether depletion affected breakdown or repair, a morphological scoring system was established; sections were assigned a score between 1 (intact decidual tissue) and 5 (complete repair) based upon key morphological features. For each animal 2-4 cross-sections from different areas of the uterus were scored blind by two independent observers. Scoring of control (n=9 per time point) and neutrophil depleted (n>15) uteri revealed that neutrophil depletion caused some retardation of endometrial breakdown. However, most notable was the significant ($p<0.001$) delay in endometrial repair. At 48h, 59% of sections from neutrophil depleted animals were delayed compared to 11% from control animals. These findings demonstrate for the first time using an *in vivo* model, that neutrophils play an important functional role in the processes of endometrial breakdown and repair.

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HYDROXYSTEROID SULFOCONJUGATION AS A PUTATIVE DETERMINANT OF FOLLICULAR LUTEINIZATION

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The process of follicular luteinization is characterized by a marked reduction in estrogen biosynthesis. In the present study, it was hypothesized that luteinization also involves the gonadotropin-dependent induction of a gene responsible for the metabolism of the estrogen precursor dehydroepiandrosterone (DHEA). DHEA sulfotransferase (SULT2A1) is responsible for the sulfoconjugation of hydroxysteroids, thereby changing their physical properties and preventing their conversion to active estrogens. This enzyme requires the presence of a sulfonate donor molecule called 3'-phosphoadenosine-5'-phosphosulfate (PAPS), synthesized by the enzyme PAPS synthase (PAPSS).

To investigate the regulation of SULT2A1 and PAPSS during follicular luteinization, the equine preovulatory follicle was used as a model. The regulation of SULT2A1 and PAPSS mRNA was studied in preovulatory follicles isolated during estrus at 0, 12, 24, 30, 33, 36 and 39 h (n = 4-6 follicles/time point) after an ovulatory dose of human chorionic gonadotropin (hCG), and in corpora lutea (n = 3) obtained on day 8 of the estrous cycle. Results from RT-PCR/Southern blot analyses showed significant changes in steady-state levels of both SULT2A1 and PAPSS mRNA after hCG treatment ($P <= 0.05$). Levels of SULT2A1 were low in follicle wall samples prior to hCG treatment and markedly increased in samples obtained at 36 h post-hCG. When analyses were performed on isolated cell preparations, a marked and significant increase in SULT2A1 mRNA was observed 33-39 h post-hCG in granulosa cells ($P <= 0.05$) and 30-39 h post-hCG in theca interna ($P <= 0.05$). Levels of PAPSS mRNA did not significantly change in intact follicle wall samples. Nonetheless, when analyses were perfomed on isolated cell preparations, a significant increase in PAPSS transcript was observed at 39 h post-hCG in granulosa cells ($P <= 0.05$), with a transient increase occuring at 12 h post-hCG in theca interna ($P <= 0.05$).

Collectively, these results demonstrate the gonadotropin-dependent induction of SULT2A1 and PAPSS mRNA in preovulatory follicles after hCG treatment and suggest that the process of follicular luteinization involves not only the downregulation of genes responsible for estrogen biosynthesis, but also the upregulation of genes involved in the metabolism of an estrogen precursor.

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NOVEL LEUKEMIA INHIBITORY FACTOR ANTAGONIST BLOCKS BLASTOCYST IMPLANTATION IN THE MOUSE

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Blastocyst implantation is a critical stage in the establishment of pregnancy. Endometrial leukemia inhibitory factor (LIF) is essential for implantation in the mouse (1), with expression peaking on day 3 (d3) of pregnancy (d0 = plug detection) in the uterine glandular epithelium (2). We tested the effect of a novel LIF antagonist on implantation in the mouse. The potent LIF antagonist MH35-BD (3) was conjugated to polyethylene glycol (PEG) to increase its half-life in vivo. The bioactivity of PEG-MH35-BD was tested using a Ba/F3 cell proliferation assay. Its in vivo half-life in mice was determined by giving a single intraperitoneal (IP) injection (1 mg/kg) of either PEG-MH35-BD or the control PEGylation reagent (mPEG2-NHS), and assaying serum at time points up to 5 days using a mouse LIF ELISA (n = 2/group). PEG-MH35-BD was present in serum from 10 mins to 5 days after injection. There was no detectable LIF in serum from control or untreated mice at any time point. Mated mice (n = 5-6/group) were injected IP with 250 µg (12.5 mg/kg) of either PEG-MH35-BD or mPEG2-NHS on d2 (12 midday and 10pm) and d3 (10am), and the uterus examined for implantation sites on d6. Only mice with visible corpora lutea at d6 were included in the study (n = 4-5/group). No implantation sites were observed in the uteri of PEG-MH35-BD-treated mice, while the control mice had normal numbers of implantation sites (0 (PEG-MH35-BD) vs. 8.8 ± 0.5 (mPEG2-NHS), mean ± SEM). Implantation was not blocked when PEG-MH35-BD was injected IP at a lower dose (6.25 mg/kg) at the same time points, or when the same doses were given 24 hours earlier on d1 and d2. These data demonstrate that a novel PEGylated LIF antagonist completely blocks blastocyst implantation in mice, providing valuable information for the development of new contraceptives for women.

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LASER CAPTURE MICRODISSECTION AND ARRAY ANALYSIS OF ENDOMETRIUM IDENTIFY CCL16 AND CCL21 AS EPITHELIAL - DERIVED INFLAMMATORY MEDIATORS ASSOCIATED WITH ENDOMETRIOSIS

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Endometriosis is an inflammatory condition. Therefore chemokine secretion in endometriosis may offer a novel area of therapeutic intervention. We aimed to identify chemokines differentially expressed in epithelial glands in eutopic endometrium from normal women and those with endometriosis and to establish the expression profiles of key chemokines in endometriotic lesions. Laser capture microdissected epithelial glands from endometrial eutopic tissue from women with and without endometriosis, in the mid-secretory phase of the menstrual cycle, were profiled using a human chemokine and receptor cDNA array. Verification of selected chemokine gene expression used real-time PCR and immunohistochemistry was also performed on ectopic endometriotic lesions. 22 chemokine and receptor genes were markedly upregulated while two were downregulated in endometrial epithelium of women with endometriosis compared with controls. Verification studies complemented this observation and statistical significance was achieved in some instances. Real-time PCR analysis of CCL16 and CCL21 demonstrated increased endometrial CCL16 but not CCL21 expression levels in women with endometriosis compared to controls (P=0.049). Immunostaining for CCL16, not CCL21, was more intense in glands in endometriosis eutopic tissue compared to controls (P=0.001). Furthermore increased CCL21 protein expression was apparent in ectopic lesions compared with matched eutopic tissue in the endometriosis patients (P=0.002). This study provides novel candidate molecules and suggests a potential local role for CCL16 and CCL21 as contributing factors towards endometriosis-related pain and infertility.

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HORMONAL REGULATION AND CONVERTASE ACTIVITIES OF COMPLEMENT 3 IN HUMAN OVIDUCTAL EPITHELIAL CELLS

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Human oviduct cells produce complement 3 (C3). The derivative of C3, iC3b, but not C3 enhanced mouse preimplantation embryo development. We hypothesized that the human oviduct uses the complement pathways for complement activation to convert C3 to iC3b via C3b and that production of C3 in the oviducts is under hormonal regulation. The aim of this study is to investigate the effect of hormones on C3 mRNA expression and the conversion of C3 into C3b/iC3b in the human oviductal epithelial cells (OE).

In vitro cultured primary OE cells were treated with varies concentrations of estrogen and progesterone either alone or in combination. Estrogen enhanced C3 mRNA expression of OE cells. Progesterone alone has no effect on C3 expression. The presence of the components of C3 convertases were studied by RT-PCR and immunocytochemistry, respectively. Molecules involved in complement activation, C2 and C4 in the classical pathway and lectin pathway, and factor B (fB) and factor D (fD) in the alternative pathway was detected in the OE cells. The OE cell culture possessed active C3 convertase that converted exogenous C3 into C3b in a time-dependent manner. No iC3b was produced under this condition.

In conclusion, the production of C3 in oviduct is estrogen-regulated and the oviductal cells can convert C3 toC3b but not iC3b.

Acknowledgements: This work is supported by Research Grant Council, Hong Kong (HKU7319/ 01M , HKU 7411/ 04M).

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THE ROLE OF IMATINIB IN THE REGULATION OF GRANULOSA CELL TUMOUR CELL GROWTH

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Granulosa cell tumours (GCTs) of the ovary are rare, hormonally active neoplasms characterised by endocrine manifestations, an indolent course, and late relapse. Chemotherapy and hormonal therapy have proved to be of limited efficacy. Highly potent, selective inhibitors of tyrosine kinases are being developed as alternatives to standard chemotherapy. Imatinib mesylate (Gleevec) is routinely used to treat chronic myelogenous leukaemia, and has recently been used successfully to treat a patient with GCT. To evaluate whether imatinib might have a role in the treatment of GCT, we sought to: 1) determine the effect of imatinib on two GCT-derived cell lines; and 2) characterise the pattern of gene expression for targets of imatinib and to screen for known activating mutations. Treatment of both cell lines with

increasing concentrations of imatinib reduced both COV434 and KGN cell proliferation and viability. In KGN cells, this was due to a dose-dependent, imatinib-induced apoptosis. COV434 cells appear to undergo necrosis. Gene expression patterns of c-ABL, c-KIT, PDGFR-α and -β were determined in a panel of GCT (n=15) and normal ovary (n=10). Uniform expression of c-ABL, c-KIT, PDGFR-α and variable expression of PFGFR-β was observed in normal ovary. Variable expression of c-ABL and both PDGFR was observed in GCT, c-KIT expression was lower than that seen in normal ovary. All genes were expressed in KGN cells; in contrast, only c-ABL was expressed in COV434 cells. Known activating mutations in c-KIT and PDGFR-β were not observed. Our data shows that imatinib can arrest proliferation in GCT-derived cells; the mechanisms involved, however, appear to differ between the two cell lines. Given that high doses of the drug caused this effect, it is likely that imatinib is exerting "off-target" effects. Although imatinib is unlikely to be effective, a tyrosine kinase inhibitor of differing specificity may be able to treat this disease.

Supported by The Granulosa Cell Tumor of the Ovary Foundation (San Diego, CA, USA) and Novartis.

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NOVEL VARIANTS IN HUMAN GDF9 IN MOTHERS OF DIZYGOTIC TWINS

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Genetic factors contribute to an increased chance of having dizygotic (DZ) twins. Genes from the ovarian bone morphogenetic signalling pathway (GDF9 and BMP15) are critical for normal human fertility. We previously identified a deletion mutation in GDF9 in sisters with spontaneous DZ twins, but the prevalence of rare GDF9 variants in twinning families is unknown. We therefore screened for rare variants in GDF9 in families with a history of DZ twinning. The GDF9 gene was screened in 279 unrelated mothers of DZ twins by denaturing high performance liquid chromatography (DHPLC). Variants were confirmed by DNA sequencing and selected variants typed by MALDI-TOF mass spectrometry in 3376 individuals from 923 DZ twinning families (2317 mothers of twins) and in 1512 controls of Caucasian origin. We found two novel insertion/deletions (c.392-393insT, c.1268-1269delAA) and four missense alterations in the GDF9 sequence in mothers of twins. Two of the missense variants (c307C>T, p.Pro103Ser and c.362C>T, p.Thr121Leu) were located in the pro-region of GDF9 and two (c.1121C>T, p.Pro374Leu and c.1360C>T, p.Arg454Cys) in the mature protein region. For each variant, the frequencies were higher in cases compared to controls, with significant differences for c.1268-1269delAA , p.Pro103Ser and p.Pro374Leu. The frequency of all GDF9 variants was significantly higher ($P<0.0001$) in mothers of twins (4.12%) compared with controls (2.29%). We conclude that rare variants altering the GDF9 protein sequence are significantly more common in mothers of DZ twins than controls suggesting that GDF9 variants contribute to the likelihood of dizygotic twinning.

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A NOVEL MODE OF ACTION FOR OXYTOCIN AND cAMP IN REGULATING MYOMETRIAL CONTRACTILITY

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Background: The heat shock proteins (HSP) 27 and 20 are implicated in modulating vascular smooth muscle contractility. Once phosphorylated, HSP27 changes conformation, thus facilitating actin/myosin interaction. Conversely, phosphorylation of HSP20 relaxes smooth muscle. The role of these HSPs in human myometrium has not been examined. Recently, we demonstrated an increase in total HSP27 protein through human gestation and an increase in phospho-HSP27-serine15 with gestation and labour.

Several contractile agonists phosphorylate HSP27 in vascular smooth muscle via an IP3-P38MAPK pathway. Oxytocin may act similarly in myometrium. Phosphorylation of HSP20 is cyclic nucleotide dependent. Rolipram, a cAMP phosphodiesterase inhibitor, is an effective myometrial relaxant and thus we have examined its ability to phosphorylate HSP20.

Hypotheses: Oxytocin induces phosphorylation of HSP27 whereas Rolipram induces HSP20 phosphorylation in human myometrium

Aims: To examine in human myometrial strips *in vitro*, the effects of Oxytocin and Rolipram on: 1) contractility, 2) HSP27 phosphorylation and 3) HSP20 phosphorylation.

Methods: Myometrium was obtained at elective Caesarean section. Tension of spontaneously contracting myometrial strips was measured following exposure to Oxytocin 10^{-7}M or Rolipram (10^{-10}-10^{-6}M). Following treatment, protein

from myometrial strips was extracted and resolved by 1D and 2D SDS-PAGE. Western blotting was performed using antibodies for total HSP20, total HSP27 and the phosphorylated forms: ser15, ser78 and ser82.

Results: Oxytocin caused a significant increase in phospho-HSP27-ser15 (n=4, $P<0.05$). associated with a 141 ± 11% increase in spontaneous myometrial contractility. Phospho-HSP27-ser-78 and 82 were unchanged. Oxytocin did not change phosphorylation of HSP20. Rolipram caused significant increase in phospho-HSP20 (n=4, $P<0.05$), associated with an 84 ± 7% reduction in contractility; there was no change in total HSP27 or its phospho-isoforms.

Conclusion: Oxytocin stimulated contractions induce a specific phosphorylation of HSP27 on serine15. Rolipram induced relaxation increases phosphorylation of HSP20. This data provides support for a novel role for phosphorylated HSP27 and 20 in modulating contraction and relaxation in human myometrium.

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GROWTH RESTRICTED FETAL AND NEWBORN RATS HAVE ALTERED BRAIN NEUROSTEROIDS

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The neurosteroid allopregnanolone is an agonist at the $GABA_A$ receptor and suppresses CNS activity. Previous studies have shown that allopregnanolone has potent neuroprotective actions in models of hypoxia-induced brain injury. We have shown that allopregnanolone concentrations are high during fetal life, decline rapidly after birth, and increase in the fetal and neonatal brain in response to acute hypoxia. We proposed that availability of adequate precursor is key to generation of this protective response, and that the growth restricted newborn may be vulnerable to hypoxic brain damage due to a reduced capacity to produce neurosteroids. Our aim was to investigate allopregnanolone concentrations in the fetal and neonatal brain from normal and growth restricted pregnancies. Bilateral uterine artery ligation (or sham control) was performed at day 18 of gestation (term=d22) to induce *in utero* growth restriction in Wistar Kyoto rats. Allopregnanolone was extracted from frozen brains from d20 fetuses and d6 postnatal rats and measured by radioimmunoassay (expressed as nmol/g protein). At d20 brain allopregnanolone concentrations were *higher* in growth restricted fetuses (2.30 ± 0.21 nmol/g, n=6) compared to control fetuses (1.37 ± 0.10 nmol/g n=6) ($p<0.05$). Allopregnanolone concentrations decreased significantly at birth in both growth restricted and control groups. However, at postnatal d6 allopregnanolone concentrations were *lower* in the brain of growth restricted pups (0.43 ± 0.02 nmol/g, n=5) compared to controls (0.65 ± 0.04 nmol/g, n=8; $p<0.05$). These findings indicate that growth restriction is a potent stimulus for neurosteroid synthesis in the fetal brain in late pregnancy. The decline in neurosteroid levels after birth may be due to the loss of precursor supply from the placenta, or to decreased enzyme activity with increased tissue oxygenation. The inability of the growth-restricted neonatal brain to produce sufficient allopregnanolone may render it particularly vulnerable to hypoxia-induced injury due to the lower neurosteroid concentrations.

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PUBERTY ONSET IS DELAYED FOLLOWING PLACENTAL AND LACTATIONAL RESTRICTION

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Accelerated growth following fetal growth restriction is implicated as contributing to increased risk of developing adult diseases which may be associated with alterations in reproductive maturation. Our aim was to determine the effects of placental restriction, the major feature of human pregnancies complicated by intrauterine growth restriction, on the timing of puberty onset. Cross-fostering at birth enabled separation of the effects of prenatal and postnatal environments on growth and reproductive maturation. Bilateral uterine vessel ligation (Restriction, R) or Sham control surgery (Control, C) was performed on day 18 of gestation in Wistar Kyoto rats. Control, Reduced (reduced litter size of Control to 5, to match Restricted) and Restricted pups were cross-fostered onto a Control or Restricted mother on postnatal day 1 to generate six groups: control pup on a control mother (C-on-C); control-on-restricted (C-on-R); reduced litter control (RED)-on-control (RED-on-C); reduced litter-on-restricted (RED-on-R); restricted-on-control (R-on-C); restricted-on-restricted (R-on-R). The day of puberty onset was the day of vaginal opening in females and the day of balano-preputial-separation in males. Data were analysed by ANOVA. Birth weights of Restricted pups were lower than Controls ($p<0.05$). R-on-R female and male pups grew slowly during lactation and after weaning while R-on-C pups accelerated their growth during lactation ($p<0.05$). R-on-R males and females had delayed day of puberty onset compared to C-on-C with the cross-fostering groups intermediate ($p<0.05$). R-on-R females had accelerated growth and body weight at puberty onset was not different between the groups. Male R-on-R pups remained small to 6 months. Placental and lactational restriction delayed puberty onset while improving postnatal growth prevented the delay. Whether the compromise of puberty onset in females by a restricted intrauterine life is associated with different

sex steroid profiles or involved in the sex differences arising in the development of adulthood diseases for small babies remains to be determined.

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RESTRICTION OF PLACENTAL GROWTH AND SIZE AT BIRTH INCREASES PANCREATIC EXPRESSION OF THE β-CELL SURVIVAL FACTOR IGF-II.

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Poor growth before birth impairs insulin secretion later in life, contributing to an increased risk of Type 2 diabetes. Poor placental growth or function is a major cause of low birth weight in humans, and we have shown that experimental restriction of placental growth (PR) in sheep (1) impairs insulin secretion from early postnatal life. Insulin secretion is determined by β-cell mass and function, and we have therefore investigated the impact of PR on expression of genes that regulate β-cell neogenesis and survival postnatally.

Expression of the β-cell survival factor IGF-II, of the β-cell and β-cell precursor marker Pdx-1, and of 18S, were measured in pancreas of 43-day old control and PR lambs. RNA was extracted from pancreas, and cDNA prepared by reverse-transcription of 2 μg RNA. Expression was measured by qPCR detection with SYBR Green, using primers for bovine ribosomal 18S and ovine IGF-II and Pdx-1. Identity of amplicons was confirmed by sequencing. Effects of PR were assessed by Mann-Whitney U-test. Data are expressed as mean ± SEM.

PR did not alter relative pancreas weight (P=0.2). PR increased expression of IGF-II (p=0.025), tended to increase expression of Pdx-1 (p=0.088), and did not alter that of 18S (p>0.1) in the pancreas of the young lamb (Table).

	Control	Placentally restricted
n	15	12
Pancreas wt (% of body weight)	0.119 ± 0.019	0.090 ± 0.009
IGF-II:18S (x 10-5)	67 ± 43	5092 ± 2818
Pdx-1:18S (x 10-5)	4.13 ± 1.52	4.92 ± 1.32

Increased pancreatic expression of IGF-II and Pdx-1 suggest that postnatal β-cell proliferation and survival may be increased following PR. Despite this, insulin secretion relative to sensitivity is impaired, implying that compensatory mechanisms to increase β-cell mass are insufficient to maintain function, possibly due to impaired intrinsic β-cell capacity.

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DIRECT EFFECTS OF ALDOSTERONE *IN VIVO* ON ENDOTHELIN-1 GENE EXPRESSION IN THE KIDNEY AND COLON

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Aldosterone facilitates sodium reabsorption from epithelial tissues such as the kidney and colon via a pathway involving activation of intracellular mineralocorticoid receptors (MRs) and subsequent regulation of target gene expression. We have investigated a putative aldosterone gene target endothelin-1 (ET-1), that encodes a 21 amino acid vasoconstrictor peptide that has been shown to affect both sodium transport and H+ secretion in the collecting duct of the kidney. There is also evidence to support a role for ET-1 in the pathogenesis of renal interstitial fibrosis. We have assessed ET-1 as a potential aldosterone-regulated gene in rat kidney and colon. Adrenalectomized adult Sprague Dawley rats maintained on 0.9 % saline were either injected with aldosterone(10ug/kg) or saline and analysed after one hour. Analysis by real time PCR indicated a 1.7 and 2.0 fold increase in ET-1 mRNA levels in the kidney and colon respectively one hour after treatment with aldosterone. Further analysis revealed no significant change in the mRNA levels endothelin-2 and -3 or the endothelin A and B receptors, one hour following treatment with aldosterone. Treatment of rats with aldosterone and potassium carenoate, a MR antagonist, blocked induction of ET-1 mRNA suggesting that these effects were mediated via MR. Finally, time course experiments showed a rapid induction of ET-1 mRNA levels after aldosterone treatment with peak levels of ET-1 mRNA reached after 1 hr in colon (2.5 fold) and 2 hrs in the kidney (5 fold) respectively. These results suggest that the endothelin-1 gene is a direct aldosterone gene target and may play an important role in mediating the action of aldosterone in the regulation of ion homeostasis.

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MATERNAL DEXAMETHASONE TREATMENT IN EARLY GESTATION SUPPRESSES STEROIDOGENIC CAPACITY AND GROWTH OF THE FETAL ADRENAL

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In most mammalian species, adrenal development is characterized by periods of active adrenal hyperplasia and steroidogenesis, which may be interrupted by periods of "quiescence" when fetal adrenal growth is substantially slowed, and steroidogenesis and responsiveness to ACTH is suppressed. In this study, we investigated the effect of a 5 day infusion of a low-dose of maternal dexamethasone (DEX: 20ug/kg maternal body-weight/d) from 56-57d gestation to inhibit fetal pituitary ACTH secretion and inhibit the expression of key markers of fetal adrenal growth (cyclin D1) and steroidogenesis (cytochrome P450 17a hydroxylase (CYP17), cytochrome P450 cholesterol side-chain cleavage (CYP11A1), 3beta hydroxysteroid dehydrogenase (3bHSD), melanocortin type 2 receptor (MC2R or ACTH receptor)). Fetal sheep adrenal glands (pairs) were collected from fetuses at 61-62d following maternal DEX (n=22) or saline-treatment (n=19), RNA extracted for analysis of CYP17, 3bHSD, CYP11A1, MC2R and cyclin D1 by Real-Time PCR and data expressed relative to ribosomal protein mRNA (RPO). Maternal DEX treatment reduced (p=0.06) fetal adrenal weight (33.9±1.7mg) compared to the saline-treated controls (39.8±2.6mg). Adrenal expression of CYP17:RP0 mRNA was reduced (p=0.004) in fetuses following maternal DEX-treatment (307.1±50.9) compared to those from the saline-treated group (621.2±79.9). In contrast to CYP17, there was no effect of maternal DEX treatment on CYP11A1, 3bHSD, MC2R or cyclin D1 expression. In summary, low-dose DEX inhibited CYP17 expression, without limiting other key steroidogenic enzymes or MC2R in the fetal adrenal gland. Interestingly, we found that low-dose maternal DEX-treatment decreased fetal adrenal weight, without inhibiting the high expression of cyclin D1 that occurs at ~60d gestation. The differential effect of DEX in the early phase of adrenal activation on the expression of the rate-limiting cell cycle protein (cyclin D1) compared with a key enzyme regulating steroidogenesis (CYP17), suggests that cyclin D1 expression may not be regulated by ACTH.

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SALIVARY CORTISOL TO MONITOR HYDROCORTISONE TREATMENT IN PATIENTS WITH HYPOADRENALISM

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Aims: To define salivary cortisol kinetics in normal subjects and assess the utility of salivary cortisol concentrations to monitor hypoadrenal patients treated with hydrocortisone.

Methods: Frequent samples of blood (13-18/subject/24hr) and saliva (40/subject/24hr), as well as a 24 hr urine, were taken from 20 control subjects and 20 patients with confirmed primary adrenal failure. All patients were on a stable maintenance dose of hydrocortisone (median 20mg/24hr, range 15–40 mg/24hr). Blood samples were assayed for total cortisol and corticosteroid-binding globulin (CBG) by ELISA and free cortisol was calculated using the method of Coolens from total cortisol and CBG. Saliva and urine cortisol concentrations were measured by ELISA.

Results: Serial saliva sample collection was well tolerated by all subjects. The mean (standard deviation) of cortisol measurements in controls and patients were:

Cortisol area under the curve (AUC)	Controls	Patients
Total plasma cortisol (0730-1730hr)	2823 (505) nmol/L.hr	3915 (808) nmol/L.hr
Calculated free plasma cortisol (0730-1730hr)	198 (69) nmol/L.hr	502 (191) nmol/L.hr
Salivary cortisol (24 hours)	238 (63.8) nmol/L.hr	1285 (850) nmol/L.hr
Urinary cortisol excretion (24 hours)	295 (160) nmol/24hrs	452 (340) nmol/24hr

The 24hr urine cortisol excretion for patients represented 0.8% (0.5%) of the daily hydrocortisone dose. In control subjects, salivary cortisol had a positive bias of 70% and a standard uncertainty of 120% relative to calculated free plasma cortisol. In hypoadrenal subjects ingestion of hydrocortisone caused contamination of saliva specimens for 1.8h (1.5), even after mouth rinsing and meal consumption. In control subjects the salivary cortisol concentration after 2200h was <10nmol/L in all cases.

Conclusions: In normal controls, salivary cortisol was an imprecise measure of free cortisol. Contamination of saliva after swallowing hydrocortisone tablets limits the utility of measuring salivary cortisol concentrations as a method of monitoring of hydrocortisone treatment.

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ELEVATED P-GLYCOPROTEIN EXPRESSION LIMITS GLUCOCORTICOID RECEPTOR RESPONSE TO CORTISOL AND IMPEDES DEXAMETHASONE TRANSPORT ACROSS MONOLAYERS IN PLACENTAL BEWO CELLS

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Studies in the rodent brain show that P-glycoprotein (P-gp) is an important physiological regulator of glucocorticoid access to the glucocorticoid receptor (GR) in target cells. Therefore, we hypothesised that placental P-gp may serve to exclude maternal glucocorticoids from the placenta and fetus, and thereby augment the placental glucocorticoid barrier and decrease the likelihood of glucocorticoid-induced fetal growth retardation. The current study used placental choriocarcinoma BeWo cells and a virally transduced daughter cell line, BeWoMDR which overexpresses P-gp. We investigated whether P-gp regulates access of cortisol to the GR in the two cell lines. Additionally, ^{3}H-dexamethasone was used to assess the rate of transport of glucocorticoids across monolayers of BeWo and BeWoMDR cells.

Elevated levels of P-gp in the BeWoMDR cells decreased the activation of the GR (P<0.05) to 30-50% of that in the parental BeWo cells across a wide range of physiological cortisol concentrations (10^{-10}M to 10^{-6}M). Diffusion of ^{3}H-dexamethasone across BeWoMDR monolayers over a period of 6 hours occurred at a slower rate (P<0.01) than across BeWo monolayers. The ^{3}H-dexamethasone diffusion was impeded in the BeWoMDR cells from as early as 2 hours. However, in the presence of cyclosporin A, a P-gp inhbitor, the diffusion rate of ^{3}H-dexamethasone from the apical to the basal chambers was identical between the two cell lines at all times.

These data support the hypothesis that P-glycoprotein contributes to the placental glucocorticoid barrier and may act in unison with 11β-HSD2 to reduce fetal and placental exposure to maternal glucocorticoids and minimise their growth inhibitory actions.

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ACTIVIN-A BINDS FOLLISTATIN AND TYPE II RECEPTORS THROUGH OVERLAPPING BINDING SITES: GENERATION OF MUTANTS WITH ISOLATED BINDING ACTIVITIES

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Follistatin is a potent extracellular antagonist of members of the TGF- β superfamily that utilize activin type II receptors (ActRII/IIB) as part of their signaling complex. A recent crystallographic study indicates that follistatin contacts activin-A residues at both the type I (ALK4) and type II receptor binding interfaces. However, the relative contribution of these two sites on human activin-A to follistatin binding has not been determined. Residues at these sites were mutated to alanine and mutants were screened for their ability to bind follistatin and ActRII and to induce FSH secretion from a gonadotrope cell line. Despite extensive mutagenesis across the type I receptor interface, activin-A affinity for follistatin was not significantly diminished. In contrast, mutagenesis of residues at the type II binding interface had pronounced effects on activin's interaction with follistatin. In particular, residues Leu^{92}, Tyr^{94}, Ile^{100} and Lys^{102} were critical for high affinity follistatin binding. Interestingly, mutation of another primary determinant of ActRII/IIB binding, Ser^{90}, did not affect follistatin affinity suggesting that the interaction surfaces for type II receptors and follistatin were overlapping but not identical. In support, mutation of Asp^{95}, on the opposite edge of the common ActRII/follistatin interface, was disruptive for follistatin binding without affecting ActRII/IIB interactions. Activin-S90A was able to compete with wild type activin for follistatin binding, whereas activin-D95A, due to its 8-fold lower affinity for follistatin, is a potent activin agonist. These reagents could be used to modulate follistatin antagonism of activin and related ligands in processes such as cancer, wound healing and reproduction.

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DIFFERENT ENVIRONMENTAL STRESSES ELICIT DIFFERENTIAL CRH RESPONSES IN LIMNODYNASTES PERONII

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Metamorphosis of Anuran tadpoles is dependent on thyroid hormone. The classic thyrotropin-releasing hormone (TRH)/ thyroid stimulating hormone (TSH)/thyroid hormone (TH) pathway, does not operate in larval frogs. However, corticotropin-releasing hormone (CRH) stimulates TSH production and may regulate TH during metamorphosis (Denver, 1997). Under desiccation stress an early rise in CRH was found in early metamorphosing desert toads.

Injection of CRH into Scaphiopus hammondii, Rana perezi and Bufo arenum induced early metamorphosis, whereas CRH receptor antagonists and anti-CRH antibodies delayed metamorphosis. This study examines CRH peptide, CRH gene expression and growth characteristics in the Australian anuran, Lymnodynastes peronii in response to water or food stress. Sibling tadpoles were randomly assigned either to one of three treatments of constant abundant water/different food availability regimes or two constant adequate food/different water volume regimes. Tadpoles were harvested at premetamorphosis (Gosner stage 30), prometamorphosis (Gosner stage 37), and climax (Gosner stage 42). Physical measurements (length, weight, age) at each stage were made. Brain CRH was measured throughout metamorphosis by radioimmunoassay (RIA). CRH gene expression was assayed using Real-Time quantitative RT-PCR. Body weight showed dependence on food availability under abundant water conditions, and on water when provided with abundant food. In abundant water, ages to stage differences were greatest to stage 37 but not significantly different at stage 42. Abundant food and water treatment tadpoles reached all stages earliest. In abundant food/different water conditions the water stressed tadpoles attained all stages earliest though significance was lost by stage 42. CRH was detected increasingly through metamorphosis in the tadpoles. Food stressed tadpoles showed a rise in CRH peptide at stage 37 and in CRH gene expression levels at climax whilst water stressed tadpoles exhibited a rise in CRH gene expression at stage 37 but no significant differences in peptide at stage 37 or stage 42. CRH involvement in the metamorphosis of Anurans may be differentially regulated according to the type of stress.

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FUNCTIONAL FSH-SECRETING ADENOMA IN MEN1

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Gonadotropinomas constitute 35% of pituitary tumours. 35% of gonadotropinomas are functional, causing high alpha-subunit and/or FSH serum concentrations [1] . 15-50% of patients with MEN1 have pituitary adenomas [2] : most are prolactinomas while some secrete alpha-subunit [3] . A case of a pituitary adenoma secreting FSH *in vitro* associated with the MEN1 syndrome was reported in 1992 [4] in a man with normal serum levels of gonadotropins. We describe a case *in vivo* of an FSH-secreting gonadotropinoma in a man with MEN1 syndrome. A 48 year-old man presented with progressively deteriorating visual acuity and bitemporal hemianopia due to a macroadenoma encircling both carotids and compressing the optic chiasm. His libido and erectile function had been reduced for two years. Serum FSH was markedly elevated at 32 IU/L [RR1-10]; alpha-subunit was elevated at 2.25 IU/L [RR0.09-0.4]; LH normal at 4 IU/L [RR1-10]; total testosterone low at 6.8nmol/L [RR9.5-35] and prolactin slightly high at 433ml/UL [RR50-300]. He had undergone parathyroidectomy for primary hyperparathyroidism in 2004. Trans-sphenoidal hypophysectomy was performed. Pituitary histopathology showed a tumour with an abundance of cells showing FSH immunoreactivity. Stereotactic pituitary radiotherapy is planned to treat the substantial residual tumour. Nevertheless, a marked visual field improvement has occurred, FSH has decreased to 16.8 IU/L and testosterone has increased to 17.2nmol/L. Libido and sexual function have improved. A family history of endocrine neoplasia was obtained of one sibling with a non-functioning pituitary adenoma, another sibling who died from pancreatic carcinoma and a third sibling, along with her son, with primary hyperparathyroidism. Genetic testing for MEN1 was performed and a nonsense mutation R460X (nt7605C>T) located on exon 10 of the MEN1 gene was found, previously described in MEN1 [5]. We believe this is the first reported case of clearly high circulating immunoreactive FSH due to a functioning FSH–secreting gonadotropinoma in the MEN1 syndrome.

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LACK OF ESTROGEN LEADS TO A SIGNIFICANT REDUCTION IN AREA AND CELL NUMBER OF A REGION CORRESPONDING TO THE SEXUALLY DIMORPHIC NUCLEUS (SDN) OF THE MEDIAL PREOPTIC AREA IN MALE AND FEMALE MICE OF THE SV129J STRAIN

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Sex differences in the developmental morphology of the mammalian brain are widely attributed to varying levels of steroid hormones. In particular, the development of the Sexually Dimorphic Nucleus (SDN) located in the medial preoptic area (MPOA). This nuclei is approximately 5-8 times larger in male than in female rats, and a positive correlation exists between SDN volume and male sexual behaviour. In the mouse, the SDN is relatively uncharacterized. In this study we use galanin and aromatase as markers to examine the mouse SDN. We have identified distinct clusters of galanin positive (gal+ve) neurons located within the MPOA which corresponded anatomically to the SDN, which will be referred to as "galanin SDN (gSDN)". Sex differences in the area of the gSDN were found in the Sv129J ($p=0.06$) strain of mice with males showing larger gSDN areas, whilst no sex differences were found in the C57/BL6 strain. Further analysis revealed significant decreases in gSDN area ($p=0.015$) in male aromatase knockout (generated by exon9 deletion of the *Cyp19* gene) (ArKO) compared to WT (Sv129J strain). In addition, significantly less gal+ve neurons were present within this area in both male ($p=0.015$) and female ($p=0.007$) ArKO compared to WT. Using *in situ* histochemistry techniques for aromatase exon 4, significant sex differences were found, with WT males exhibiting larger SDN area ($p=0.015$). In correlation with the gSDN, both male and female ArKO mice displayed smaller arom+veSDN area than same sex controls. Interestingly, male ArKO gSDN and arom+v SDN size resembled that of female WT littermates suggesting a possible feminization of this part of the brain. The male ArKO mice have previously been shown to display a total lack of sexual behaviour. The diminished size of the SDN (both markers) shown here may attribute to the disruption of sexual behaviour in the male ArKO.

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PROSTATE ATROPHY AND ABNORMAL EPITHELIAL CELL PROLIFERATION DUE TO TARGETED DISRUPTION OF THE PROSTATE EPITHELIAL ANDROGEN RECEPTOR IN PEARKO MICE

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Stromal-epithelial interactions and androgen actions via androgen receptor (AR) within these compartments are important for prostate development and maturation. However, so far it has not been possible to clearly separate the roles of epithelial and stromal AR in mature prostate. We established a novel mouse model with targeted disruption of prostate epithelial AR to selectively study the role of epithelial AR in the mature prostate. A transgenic mouse line expressing Cre under a probasin promoter Pbsn-cre were crossed with a mouse line containing a floxed AR gene, exon 3 flanked by loxP sites, to generate male progeny exhibiting a prostate epithelial specific AR knockout (PEARKO). Crossing Pbsn-cre with ROSA reporter mice revealed Cre expression in epithelia of all prostate lobes, as well as seminal vesicle and epididymis epithelium at two weeks of age. At eight weeks of age, PEARKO males had significantly ($p<0.05$) decreased weight of prostate lobes (36, 47 and 80% of control, for anterior, dorsolateral and ventral prostate, respectively), but unchanged testis weight or serum testosterone compared with Cre littermate controls. Yet, despite decreased prostate lobe weights, microdissection revealed normal branching morphogenesis of all lobes in PEARKO mice. Volumetric proportion of epithelia was significantly ($p<0.05$) decreased and that of lumen increased in PEARKO anterior (44 and 39%, respectively) and dorsolateral (49 and 26%, respectively) prostate while the volumetric proportions of epithelia, lumen and stroma were similar for ventral prostate in PEARKO and control males. The most significant qualitative changes were abnormal clustering of epithelial cells predominantly in anterior lobe but also in dorsolateral and ventral lobes of PEARKO mice. These results indicate that disruption of epithelial AR signalling and thereby normal stromal-epithelial interactions lead to abnormal morphology and growth of prosate epithelial cells. (Academy of Finland #107825 and Finnish Cultural Foundation of Northern Savo)

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ANDROSTANEDIOL AND DEVELOPMENT OF THE WOLFFIAN DUCTS IN TAMMARS

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Sexual differentiation in marsupials takes place after birth, when the young is developing in the pouch. Wolffian duct differentiation depends on androgens, but two questions are unanswered: which androgen drives this process, and how is the androgen delivered to the duct?

We have previously shown that 5a-androstanediol (5α-adiol; 5α-androstane-3α, 17β-diol) is the circulating androgen in developing males responsible for prostate and penile differentiation. Female tammar neonates treated with 5a-adiol from day 10 to day 35 retained their Wolffian ducts whilst the control treated females did not. Wolffian duct development in male neonates treated from day 10 to day 35 with 4MA, an inhibitor of 5α-adiol and DHT formation was inhibited. Unexpectedly 4MA treated males retained their Müllerian ducts. Müllerian ducts were also retained in oestrogen treated males in previous experiments, suggesting that inhibiting 5α-reductase may have allowed a build up of testosterone that was aromatised to oestradiol. We grafted neonatal testes beneath the skin of neonatal females and found that the Wolffian ducts in these females were retained, showing that testicular androgens do not need to be delivered locally to the duct, but can act via the systemic circulation.

Thus Wolffian duct development in this marsupial depends on a 5α-reduced steroid that can be delivered by the systemic circulation. The most likely candidate is 5a-androstanediol.

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ANTI-ACTIVIN CONSEQUENCES OF GLUCOCORTICOID ACTION WITHIN THE MALE REPRODUCTIVE AXIS

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Local activin actions are partly determined by follistatin (FS), inhibin and its co-receptor, betaglycan (BG). Inhibin binding to BG promotes its antagonism of activin actions through the sequestration of the type II receptors. Glucocorticoids are known to increase the expression of BG and FS in some non-reproductive tissues. Since gonadotrophs, Leydig cells and primary spermatocytes express glucocorticoid receptor (GR), we hypothesized that glucocorticoids inhibit activin actions in reproductive tissues through the up-regulation of BG and FS expression.

Mouse Leydig (TM3), gonadotroph (LβT2) and germ line [GC-2spd(ts)] cells, but not Sertoli (TM4) cells, were found to express GR, and all cell lines expressed BG and FS. Overnight treatment of these cells with the synthetic glucocorticoid, RU28362 (250 nM), gave the following tabulated changes (% of the matching vehicle-treated control in each case in [*n*] independent experiments) in their relative BG and FS mRNA levels , and [^{125}I]inhibin A binding to whole cells: [*, ** P<0.05, P<0.01 relative to 100%]

Cell line	BG mRNA level	FS mRNA level	[^{125}I]inhibin A binding
TM3	285 ± 19** [*5*]	500 ± 60** [*5*]	134 ± 3** [*9*]
TM4	113 ± 2* [*6*]	81 ± 10 [*4*]	121 ± 10 [*4*]
LβT2	222 ± 23** [*6*]	189 ± 40 [*4*]	126 ± 3** [*5*]
GC-2spd(ts)	139 ± 9* [*3*]	149 ± 17 [*3*]	133 ± 4* [*3*]

As a representative cell line, TM3 cells were transfected with the activin-responsive pGRAS-luc reporter. Treatment of cells with RU28362 decreased the inhibin A IC50 for antagonism of activin A-stimulated luciferase expression from 190 to 77 pM.

In summary, glucocorticoid treatment increased BG expression, promoted its interaction with inhibin A, and stimulated follistatin expression in cell lines modelling gonadotrophs, Leydig cells and spermatocytes, but not Sertoli cells, consistent with the pattern of GR expression by these cell types. In TM3 cells, glucocorticoids concomitantly increased inhibin potency. Thus, glucocorticoids display dual anti-activin actions in multiple responsive cell types within the male reproductive axis.

Funded by the NH&MRC of Australia (RegKey 241000 & 198705)

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CALMODULIN-DEPENDENT NUCLEAR IMPORT OF THE TESTIS-DETERMINING FACTOR SRY

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Sex determination in mammals is determined by the chromatin-remodeling factor, SRY (Sex determining Region on the Y chromosome). SRY is expressed embryonically in Sertoli cell precursors and, through its high mobility group (HMG)- box domain, acts as a switch within the nucleus at specific DNA targets to modulate gene expression, leading to the development of the testis. A number of mutations in SRY that result in human XY sex reversal map to one of SRY's two independently acting nuclear localisation signals (NLSs) that flank its DNA binding domain. The C-terminal NLS (C-NLS) targets SRY to the nucleus through the conventional nuclear import receptor importin-β1 (Imp-β1), but no importin has been shown to bind the N-terminal NLS (N-NLS), although it is known to interact with the Ca^{2+}-binding protein calmodulin (CaM). In this study, we examine various missense mutations in the SRY N-NLS from XY sex-reversed females for effects on nuclear import and ability to interact with CaM and Imp-β1. The mutations were all found to result in reduced nuclear localisation in transfected cells compared to wild type. The CaM antagonist, calmidazolium chloride (CDZ), was found to significantly reduce SRY nuclear accumulation, indicating dependence of SRY nuclear import on CaM. Intriguingly, N-NLS mutants were resistant to CDZ's effects, implying a loss of interaction with CaM, which was confirmed directly by *in vitro* binding experiments. These results strongly implicate a CaM-dependent nuclear import pathway for SRY mediated by the N-NLS that, together with the C-NLS, is required to achieve threshold levels of SRY in the nucleus for male sex determination during foetal development.

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LIVER RECEPTOR HOMOLOGUE-1 (LRH-1) REGULATED GENES WITHIN THE TESTIS.

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LRH-1 is an orphan nuclear receptor localised in the Leydig and germ cells. It has been associated with regulating cell proliferation and tumourgenesis, but its function within the testis has yet to be elucidated. We have demonstrated that LRH-1 has the ability to regulate aromatase within cells isolated from adult rat testes, however little is known about other LRH-1 regulated genes. Therefore we aimed to identify potential LRH-1 regulated genes by two complementary methods. Affymetrix microarrays and a database of proximal promoters (PAGEN@UIC) were utilised to ensure the genes of interest are both potentially regulated by LRH-1 and possess the LRH-1 consensus DNA binding sequence (CAAGGTCA). Affymetrix microarray analysis was performed on primary Leydig and germ cells infected with a full length LRH-1 recombinant adenoviral construct to over express LRH-1, to determine genes that are either up or down regulated in the presence of LRH-1. The Affymetrix microarray analysis generated a list of 200 genes for each cell type that were regulated by LRH-1. Nine genes were selected and identified to contain the consensus LRH-1 DNA binding site within their promoter region (SOCS1, BMP4, IGFBP5, FABP9, Wifl, Foxa2, tsaga13, Gdf10, IL6, Klf5 and Kit ligand). There were five other genes of interest regulated by LRH-1 which did not appear to contain the consensus sequence in their promoter region, but may be indirectly regulated by LRH-1. Genes which did not appear to be regulated by LRH-1, but contained the consensus LRH-1 sequence were also identified as they may potentially be important. Many of the identified genes are known to be involved in cell cycle and development and they may have important, but as yet unknown roles in the testis. Work is currently being performed to validate the selected genes within the different cells and to determine their role in testicular physiology.

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ACTIVIN βC-SUBUNIT IS A REGULATOR OF TESTIS AND LIVER FUNCTION: IMPLICATIONS FOR ACTIVIN BIOLOGY

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Dimeric activins (βA βA , βB βB) are growth and differentiation factors with potent *in vivo* activities in diverse biological systems including mesoderm induction and early embryogenesis. The related activin βC subunit forms homodimers as well as heterodimers with βA and βB subunits *in vitro*, but appears to have little functional significance based on lack of abnormalities in activin βC-subunit knock-out mice. We proposed the activin βC-subunit is a functional antagonist of activin A, forming heterodimers in tissues that co-express other activin subunits. To test this hypothesis we generated activin βC-subunit over-expressing mouse lines and observed pathologies in testis and liver. Activin βC-subunit over-expression reduces circulating activin A levels and male transgenic mice develop a

progressive, age-related, decrease in fertility. A decline in litter sizes was associated with reduced sperm output due to a stage-specific increase (stages V-VIII and IX-XI) in apoptosis during spermatogenesis and impaired sperm motility. Further, the livers of transgenic mice were enlarged due to an imbalance between hepatocyte proliferation and apoptosis; foci of inflammatory cells were evident which were associated with significant changes in liver enzymes. The data demonstrate that in tissues in which activin βC-subunit expression is up-regulated, activin biology is impaired leading to pathologies as demonstrated in the liver and testis. Collectively our data suggest the activin βC-subunit is a novel regulator of activin bioavailability. These data have implications for activin biology in any tissue in which βA and βC-subunits are co-expressed.

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GLYCOSYLATED FORMS OF HUMAN INHIBIN A AND B SHOW MARKED DIFFERENCES IN *IN VITRO* BIOACTIVITY

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Inhibin A and B are dimeric gonadal proteins consisting of an α and either βA or βB subunits. In contrast to inhibin A, the structure function characteristics of inhibin B are poorly described. The aim of this study was to purify and characterise glycosylated 31 and 34k forms of recombinant human inhibin A and inhibin B. Inhibin A and B were purified from conditioned culture medium using anti-inhibin α subunit immunoaffinity chromatography and RP-HPLC. The masses of the purified inhibin preparations were determined by specific inhibin A and B ELISAs and their *in vitro* bioactivities determined by *in vitro* bioassay based on FSH suppression in rat pituitary cells in culture. The specific bioactivities (expressed as a ratio of *in vitro* bioactivity to immunoactivity; B/I ratio) of inhibin A and B and their glycosylated forms were then determined. The mono-glycosylated 31k inhibin A was 4-5 times more potent than the di-glycosylated 34k inhibin A, (B/I ratio 1.18±0.16 (mean±sd, n=3-8) vs 0.25±0.05, $p<0.001$, resp). Deglycosylation of 31k inhibin A resulted in a B/I ratio of 2.75±0.58 suggesting that glycans play an inhibitory role in their biological activities. Similarly, the 31k inhibin B was significantly ($p<0.001$) more potent (0.91±0.21) than the 34k form (0.51±0.1). However, de-glycosylation of the 31k inhibin B showed a decrease (0.54±0.10) in B/I ratio. Note that the bioactivities of inhibin A and B were broadly similar. It is concluded that the 31k and 34k mol wt forms of both inhibin A and B differ in their bioactivities and that human inhibin preparations with differing proportions of the 31 and 34k inhibin forms will show differences in function. These findings may have implications in the physiology of inhibin if the proportions of the 31/34k inhibin forms produced are regulated.

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IN VIVO REGULATION OF TIGHT JUNCTION PROTEINS BY GONADOTROPHINS IN THE ADULT DJUNGARIAN HAMSTER TESTIS

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The blood-testis barrier (BTB), formed by binding between tight junction (TJ) proteins (occludin, claudin-11, claudin-3 and junction adhesion molecule) between Sertoli cells is essential for spermatogenesis[1]. This study aimed to assess the hormonal regulation of testicular TJ proteins *in vivo* using the adult Djungarian hamster, in which gonadotrophins and spermatogenesis cycle naturally between normal and deplete states. Long day (LD) photoperiod (16L:8D) adult hamsters were exposed to short day (SD) photoperiod (8L:16D) for 11 weeks to suppress gonadotrophins and spermatogenesis and then received 6 I.U FSH (2-10 days)[2] to re-initiate spermatogenesis[3]. Testes were Bouin's-fixed for immunohistology (claudin-3, claudin-11 and occludin) or had RNA extracted for TJ mRNA quantitation by real-time PCR.

Claudin-11 and occludin in the LD hamster were localised to the basal aspect of Sertoli cells consistent with the BTB. TJ proteins were disorganized in the SD hamster and localised principally to Sertoli cell cytoplasm. After 2 days of FSH replacement TJ proteins were reorganised, and resembled the LD phenotype by 10 days. Claudin-3 (expressed in forming TJs[4]) was not expressed in LD Sertoli cells but was localised to apical cytoplasm and around germ cells in SD animals. After two days of FSH treatment, intense claudin-3 reactivity localised to basal aspects of Sertoli cells, consistent with forming BTB. Immunoreactivity progressively decreased by 10 days treatment. In contrast claudin-11 was maximal and resembled the LD phenotype at this time. Compared to LD hamsters, TJ mRNA levels (claudin-3, claudin-11, occludin,) were increased (2 fold, $p<0.05$) in SD animals where the BTB is known to be non-functional[5].

We conclude that testicular TJs are regulated by FSH in the Djungarian hamster, as evidenced by the disorganisation of TJ protein in the SD animal and reorganisation after FSH treatment. High TJ mRNA levels and immunoreactivity in the SD hamster suggest that poorly organised TJ proteins are still present but not functional. The initiation of claudin-3 expression after gonadotrophin suppression and transient expression during BTB formation supports the hypothesis that claudin-3 is a component of newly forming TJ in the testis.

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CROSSTALK BETWEEN THE CHICKEN OVALBUMIN UPSTREAM PROMOTER TRANSCRIPTION FACTORS (COUP-TFS) AND LXR IN SKELETAL MUSCLE CELLS REGULATES LIPID HOMEOSTASIS

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ABSTRACT: The Chicken Ovalbumin Upstream Promoter-Transcription Factors (COUP-TFs) are orphan nuclear receptors that are involved in organogenesis and neurogenesis. However, their role in metabolism and major mass peripheral tissues remains unclear. Skeletal muscle is a peripheral major mass tissue that accounts for ~ 40% of total body mass and energy expenditure. Moreover, this lean tissue is a primary site of glucose and lipid utilization. Exogenous and stable expression of a COUP-TF-siRNA in a skeletal muscle cell culture model attenuated COUP-TFI and II mRNA and protein levels. This induced significant modulation of UCP1 mRNA expression (a gene involved in energy expenditure and thermogenesis) and a significant increase in ATP levels. Moreover, the expression of genes that control fatty acid homeostasis (including UCP3, PPAR a , FABP3 and CPT1) were considerably attenuated. Furthermore, repression of COUP-TFI and II suppressed the activation of the 'classical' LXR target genes (and reverse cholesterol transporters), ABCA1, and ABCG1 by the synthetic and selective LXR agonist, T0901317. In agreement, increased levels of total cholesterol were found in the COUP-TF-siRNA transfected cells. Moreover, the ability to efflux cholesterol folllowing T0901317 treatment was almost completely blunted in the COUP-TF siRNA transfected cells in contrast to the negative control cells. Concordantly, we observed that COUP-TF-II directly regulated the ABCA1 promoter. In conclusion, these data suggest COUP-TFs control lipid homeostasis, and are involved in crosstalk with LXR in the control of cholesterol homeostasis.

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CHROMATIN STRUCTURE AND NFKB BINDING IN THE PROSTAGLANDIN ENDOPEROXIDE H SYNTHASE (PGHS-2) PROMOTER IN TERM FETAL MEMBRANES

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In late pregnancy PGHS-2 expression increases in the fetal membranes leading to the production of prostaglandins that initiate labour. The mechanisms that regulate PGHS-2 gene activity *in vivo* are unknown, but changes in chromatin structure and binding of the transcription factor NFκB may play a role. This study looked at chromatin structure in the PGHS-2 promoter of term amnion and chorion to define the region(s) accessible to transcription factor binding. We also examined the possibility that changes in chromatin structure influence NFκB binding, enabling the NFκB system to regulate PGHS-2 gene activity *in vivo*. Amnion (n=5) and chorion (n=5) tissues were collected from women before and after labour. Chromatin structure was examined using chromatin immunoprecipitation (ChIP) with antibodies specific for acetylated histones H3 and H4, and PCR primers designed to scan the first 2500 bp of the PGHS-2 promoter. Increased histone acetylation indicates a relaxed chromatin structure which allows transcription factor binding and transcriptional activation. NFκB binding was determined by ChIP with NFκB (p65 and p50) and TATA-binding protein (TBP) antibodies and primers targeting consensus regions in the PGHS-2 and IκB promoters. IκB was used as an NFκB-responsive positive control gene. Both the amnion and chorion showed increased acetylated histones H3 and H4 in the first 1,000 bp of the PGHS-2 promoter. This region contains the two NFκB binding sites however; significant binding of NFκB occurred only in the amnion before labour and was not related to TBP binding to the PGHS-2 TATA-box. NFκB binding on the IκB gene was significant after labour and correlated with TBP binding. Our data suggests PGHS-2 gene transcription is regulated through a region in the first 1,000 bp of the promoter. NFκB did not appear to regulate PGHS-2 gene activity, despite an open chromatin structure, suggesting additional gene specific regulation in term fetal membranes *in vivo.*

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INTERACTIVE EFFECTS OF FETAL PROGRAMMING AND POSTNATAL DIETARY OMEGA-3 FATTY ACIDS ON METHYLATION STATUS OF RENAL GLUCOCORTICOID RECEPTOR AND 11β-HYDROXYSTEROID DEHYDROGENASE 2.

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Fetal programming is a key determinant of the adult phenotype and can induce permanent alterations in gene expression, including modifications involving DNA methylation status. We recently established that postnatal ingestion of omega-3 fatty acids attenuate aspects of glucocorticoid-induced programming, including altered gene expression (Wyrwoll *et al.*, *Endocrinology* 2006 147 :599-606). We investigated the effects of fetal glucocorticoid exposure and a postnatal diet rich in omega-3 fatty acids on the methylation status and expression of renal glucocorticoid receptor (GR) and 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) in 6 month-old offspring. Dexamethasone was administered to pregnant rats (0.75 µg/ml in drinking water) from day 13 to term. The offspring of treated and control mothers were cross-fostered to mothers on either a standard or high omega-3 diet, and remained on these diets post-weaning. In six month old offspring, renal GR and 11β-HSD2 mRNA expression was determined by real time quantitative RT-PCR and methylation status by methylation-sensitive PCR. Renal GR mRNA was elevated in both male and female offspring exposed to dexamethasone *in utero*, and this was associated with reduced methylation of the GR gene. Postnatal diet had no effect on either GR mRNA expression or methylation status. Fetal dexamethasone exposure markedly reduced renal 11β-HSD2 expression in male rats raised on a standard diet, but this effect was not observed when offspring were raised on a diet high in omega-3 fatty acids. 11β-HSD2 methylation did not differ amongst the groups. Our data show that excess glucocorticoid exposure *in utero* induces permanent alterations in renal GR expression that are associated with reduced methylation status. In contrast, programmed changes in renal 11β-HSD2 expression were not due to altered methylation. Moreover, attenuation of glucocorticoid programming effects by omega-3 fatty acids is not due to altered methylation status.

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COMPLEX INTERATIONS BETWEEN SLIRP, A SRA-BINDING NUCLEAR RECEPTOR COREPRESSOR, AND OTHER NUCLEAR RECEPTOR COREGULATORS

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SRA (Steroid Receptor RNA Activator)1 is an RNA coactivator that plays an important role in the transactivation of nuclear receptors (NRs), including the estrogen receptor (ER). SRA expression is aberrant in many human breast tumours suggesting a potential role in pathogenesis. We have previously identified a novel protein SLIRP (SRA stem-Loop Interacting RNA-binding Protein)2, from a human breast cancer cell library, binding to a SRA stem loop (STR7) 3. SLIRP is expressed in normal and tumour tissues and contains an RNA recognition motif (RRM). In this work we show that SLIRP represses transactivation of a range of NR (ER, GR, AR, VDR, TR and PPARδ). In addition, SLIRP augments the repressive effect of Tamoxifen and ICI182780 and modulates association of SRC-1 (a NR coactivator) with SRA *in vivo*. In ChIP studies, SLIRP's recruitment to endogenous E2-regulated promoters is SRA-dependent. In gel-shifts we demonstrate that binding of SLIRP to STR7 is highly specific and significantly reduced when STR7 is mutated. Mutation of SLIRP's RRM abolishes the binding of SLIRP to STR7 in gel-shifts and its repression activity in transfections. SLIRP shares sequence homology with several other published SRA-binding proteins, we investigate these in REMSA. SLIRP augments the repressive activity of SHARP, another NR corepressor. Interestingly, SKIP (a NR coregulator) is colocalized with SLIRP on human chromosome 14. SLIRP and SKIP have opposing effects on ER-mediated reporter activity. When SLIRP expression is reduced with siRNA, NCoR (a NR corepressor) recruitment is reduced while ER is recruited, suggesting SLIRP may play an important role in facilitating NCoR's function. Imaging studies confirm that the majority of endogenous SLIRP resides in the mitochondria. Taken together, our data demonstrates that SLIRP coregulates a broad range of NRs, suggests it may play a role in energy metabolism and lipid homeostasis, and provides insight into interactions between key coregulators.

(1) Hatchell et al, ESA 2004, abstract OFC-101.
(2) Lanz RB, et al. PNAS 2002, 99:16081-6.
(3) Lanz RB, et al. Cell 1999, 97: 17-27.

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cAMP REGULATES CRH GENE EXPRESSION THROUGH A MULTI-ELEMENT RESPONSE UNIT

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Corticotropin releasing hormone (CRH) is a neuropeptide expressed in the hypothalamic paraventricular nucleus (PVN), and involved in the stress response. CRH is also expressed in peripheral sites including the placenta. There is a progressive increase in placental CRH production throughout pregnancy with levels peaking at labour, and CRH concentrations have been proposed as a marker for predicting pre-term birth. CRH production in the hypothalamus is suppressed by glucocorticoids, whereas in the placenta it is stimulated by glucocorticoids. We hypothesize that differences in transcription regulation are due to tissue specific alterations of transcription factors. Therefore, we seek to identify key cis-acting elements and transcription factors which regulate the CRH gene.

In AtT20 cells (a model of PVN CRH production) cAMP stimulation occurs through two major elements: the cAMP response element (CRE) and the caudal type homeobox protein response element (CDXARE). Our data shows that the CRE acts as part of a cAMP response unit that includes the hybrid steroid response element (HRE), ecdysone response element (EcRE), metal-responsive transcription factor-1 response element (MTFRE), ying yang 1 response element (YY1RE) and negative glucocorticoid response element (nGRE). cAMP acts on the HRE, EcRE and MTFRE to block YY1RE mediated inhibition of the CRE. Glucocorticoids acting at the nGRE inhibit cAMP activation of the CRE. In placental cells the CRH promoter has low intrinsic basal activity and cAMP causes a modest increase in activity. Stimulation by glucocorticoids and cAMP and inhibition by estrogen and estrogen receptor alpha occurs through the CRE. Thus, in AtT20 cells multiple response elements coordinate a response to cAMP and glucocorticoids while in placental cells the CRE acts in isolation.

Although the CRE plays a pivotal role in them all, complex molecular systems operate in the different cellular environments to regulate CRH gene expression and meet specific physiological needs.

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THE PROLYL ISOMERASE FKBP52 ENHANCES GLUCOCORTICOID RECEPTOR SIGNALLING BY TARGETING A CONSERVED N-CAP PROLINE CRITICAL FOR HELIX 12 DYNAMICS

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Steroid receptors, including the glucocorticoid receptor (GR), belong to the superfamily of nuclear receptors that function as ligand-regulated transcription factors. Before ligand binding, steroid receptors assemble into heterocomplexes with several molecular chaperone components, including the major heat shock protein Hsp90 and immunophilin cochaperones such as cyclophilin 40 (CyP40), FKBP51 and FKBP52, which possess peptidyl prolyl *cis-trans* isomerase (PPIase) activity. In a yeast model, we have previously shown that FKBP52 potentiates rat GR transcriptional activity by increasing receptor hormone-binding affinity. Because FKBP52 PPIase activity is essential for this enhanced GR function, we have speculated that a proline residue within the ligand-binding domain (LBD) might be targeted for isomerisation, resulting in conformational changes optimal for ligand-induced transactivation. The aim of the present study was to identify this PPIase-sensitive proline.

The GR crystal structure shows the LBD to be assembled from twelve helices (H1 to H12). Sequence alignment of steroid receptor LBDs identified several conserved proline residues within intervening loops, as potential PPIase substrates. After substituting these prolines with alanine, we tested the mutated receptors for potentiation by FKBP52 in the yeast assay. GR mutants that were eliminated showed low basal activity in comparison to wild type GR, but were further enhanced by FKBP52. GR P768A however, was found to be transcriptionally inactive and not responsive to the presence of the immunophilin. Although our data requires confirmation, we propose that Pro768 is the PPIase-sensitive proline in the rat GR LBD. Pro768 occupies the N-cap position in H12 and resides close to residues in H12 and the adjacent loop that directly contact ligand. This conserved proline residue, and the similarly situated proline residues in other steroid receptors, may act as substrates for immunophilin cochaperones working in concert with Hsp90 to optimally position H12 within receptors prior to ligand binding.

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EVIDENCE FOR CROSSTALK BETWEEN THE ORPHAN NUCLEAR RECEPTOR, NOR-1 AND β-ADRENERGIC SIGNALLING IN SKELETAL MUSCLE CELLS

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Activation of β-adrenergic receptors (β-ARs) and signalling has been implicated in lipolysis, energy expenditure and skeletal muscle hypertrophy. The processes mediating these effects, the target gene(s), tissues(s) involved remain obscure. We have previously demonstrated that the orphan nuclear receptor Nur77 (NR4A1) is regulated by β-AR signalling and is involved in lipolysis and controls gene expression associated with lipid homeostasis. Subsequently, another group demonstrated that β-AR agonists and cold exposure induced Nur77 mRNA expression in brown adipose tissue and that NOR-1 (NR4A3) expression was super-induced by cold exposure in the nur77 null mice. In this study, we set out to determine if the other member of the NR4A subgroup expressed in skeletal muscle, NOR-1, had a distinct, redundant and/or overlapping regulatory role with respect to Nur77 in these cells. To examine the role of the orphan nuclear receptor NOR-1, we treated and observed that 30-240 minutes of β-AR agonist (isoprenaline) treatment significantly and transiently activated expression of this receptor in C2C12 skeletal muscle cells. In agreement with these observations, the activity of the NOR-1 promoter was enhanced by isoprenaline. Stable transfection of a NOR-1 siRNA expression vector (but not the negative control siRNA) into C2C12 skeletal muscle cells significantly repressed endogenous NOR-1 mRNA expression, and led to changes in the expression of genes involved in the control of lipid utilisation and the expression of myostatin (and the myostatin promoter), a negative regulator of muscle mass. In conclusion, NOR-1 mRNA expression is sensitive to β-adrenergic signalling, and is involved in gene expression controlling muscle mass and fatty acid utilisation.

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ARRESTIN SENSITIVITY OF CLASS A AND B G-PROTEIN COUPLED RECEPTORS (GPCRS) CAN BE DEFINED USING EXTENDED BRET (EBRET) IN DIFFERENT CELL TYPES

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Following agonist stimulation, most GPCRs undergo phosphorylation by GPCR kinases (GRKs) and subsequent recruitment of βarrestins (βarrs). This process terminates signalling (desensitisation) and promotes dynamin-dependent internalisation. GPCRs can be classified according to their ability to recruit βarr1 and 2. Class B GPCRs form a stable complex with both βarr1 and 2, while Class A GPCRs preferentially recruit βarr2 in a more transient manner. Thyrotropin-releasing hormone receptors 1 and 2 (TRHR1, Class B and TRHR2, Class A) provide an excellent model for studying GPCR-arrestin interactions in different cellular backgrounds (COS7 and HEK293FT cells) by means of extended bioluminescence resonance energy transfer (eBRET). How these interactions are affected by disrupting the endocytotic machinery with a dominant negative dynamin mutant (K44Adyn) has also been examined. Our results indicate that whether cells are suspended or adherent affects specific behaviour of protein-protein interactions. An early increase in the TRHR2/βarr2 BRET signal was observed in suspended COS7 cells in the presence of K44Adyn. The disruption of the steady state interaction between these proteins presumably occurs due to blocking of protein complex dissociation. In contrast, K44Adyn augments the BRET signal between TRHR1 and either βarr in a suspended state only after a substantial period (hours) and is consistent with a strong interaction largely resistant to dissociation upon internalisation. However, eBRET measurements in adherent cells show disruption of the system was not replicated. TRHR/βarr interactions also differed when compared in HEK293FT cells. A marked increase in BRET signal for TRHR1/βarr1 in the presence of K44Adyn in suspended HEK293FT cells was seen immediately. TRHR/βarr interactions differed again when HEK293FT cells were assayed in an adherent state and dissociation was seen after ~100 min for both TRHR subtypes and both βarrs. These results demonstrate the value of using eBRET and TRHRs to study class and cell type-dependency of GPCR/βarr interactions.

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THE RATE, EXTENT AND MODIFIERS OF SPERMATOGENIC RECOVERY AFTER MALE HORMONAL CONTRACEPTION: AN INTEGRATED ANALYSIS

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Background: Hormonal methods to provide safe, reliable, reversible contraception based on suppression of spermatogenesis may soon become available. The rate, extent and predictors of reversibility have yet to be systematically studied. Methods: An integrated multivariate time-to-event analysis of individual subject data provided by investigators of 30 studies published between 1990-2005 where sperm output was monitored monthly until recovery. Findings: 1549 healthy eugonadal Caucasian (two-thirds) or Asian (one-third) men aged 19-51 underwent 1283.5 man-years of treatment, and 705 man-years of post-treatment recovery. These data represent about 90% of all published subject data using androgen or androgen-progestin regimens. The median time required for sperm to recover to thresholds of 20, 10 and 3 million/ml were 3.4 (3.2-3.5, 95% CI), 3.0 (2.9-3.1) and 2.5 (2.4-2.7) months, respectively (Kaplan-Meier). Multivariate Cox analysis showed faster recovery with older age, Asian race, shorter treatment duration, shorter acting T preparations, higher baseline sperm concentration, faster suppression of spermatogenesis, and lower baseline blood LH. Typical probabilities of recovery to 20 million/ml within 6, 12, 16 and 24 months were 67 (61-72), 90 (85-93), 96 (92-98) and 100%, respectively. Recovery to lower sperm concentration thresholds (3 and 10 million/ml) paralleled recovery to 20 million/ml. Sperm morphology and motility improved with increasing sperm concentration. Interpretation: Hormonal male contraceptive regimens show full reversibility within a predictable time course. A variety of covariables influence the rate but not extent of recovery, but their effect sizes are relatively minor. These data form a crucial basis for the further safe and practical development of such regimens.

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RELATIVE MATERNAL HYPOCORTISOLISM IN HIGH RISK HUMAN PREGNANCY

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Background: Plasma cortisol concentrations during normal pregnancy reach those seen in Cushing's syndrome. Although cortisol is important in blood pressure regulation and immune function, little is known about maternal cortisol in pre-eclampsia, intrauterine growth restriction (IUGR) and gamete donor pregnancies.

Objectives: To study maternal cortisol and corticosteroid-binding globulin (CBG) concentrations prospectively in pregnancies at risk of pre-eclampsia and IUGR, including those conceived using donated gametes.

Methods: This was a longitudinal study of 93 high risk women, including gamete recipients (n=22) and 33 controls. Plasma total and free cortisol and CBG concentrations were measured 2-weekly from 16 weeks gestation until delivery.

Results: Within the high risk group, 42% had complications including pre-eclampsia (n=11), gestational hypertension (n=16) and small for gestational age (SGA) neonates (n=12). 32% of women with assisted reproduction by gamete donation (GD) had complications. In the hypertensive group, total and free cortisol and CBG tended to be lower from early pregnancy compared to controls, and were significantly lower in the last 6 weeks of pregnancy. In GD, plasma CBG were suppressed by approximately 20% from 20 weeks gestation until delivery, with a corresponding decrease in total and free cortisol concentrations. In IUGR, plasma CBG was lower throughout gestation, but there was no significant difference in plasma total and free cortisol. The relatively low correlation between free cortisol using Coolens' method and measured free cortisol suggests an altered binding affinity of CBG for cortisol in pregnancy.

Conclusions: Maternal plasma corticosteroid-binding globulin, total and free cortisol concentrations are reduced in pre-eclampsia and gestational hypertension, and markedly reduced in recipients of donated gametes. Low cortisol concentrations are paradoxical in pre-eclampsia where inflammatory cytokines may be expected to increase cortisol release. Low maternal cortisol may be due to a lack of placental CRH and/or ACTH driving cortisol production and may influence the foetal hypothalamic-pituitary-adrenal axis and disease later in life.

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RATE OF CHANGE OF CORTICOTROPHIN RELEASING HORMONE AND HCG NADIR PROVIDE ACCURATE IDENTIFICATION OF WOMEN AT RISK OF PRETERM BIRTH

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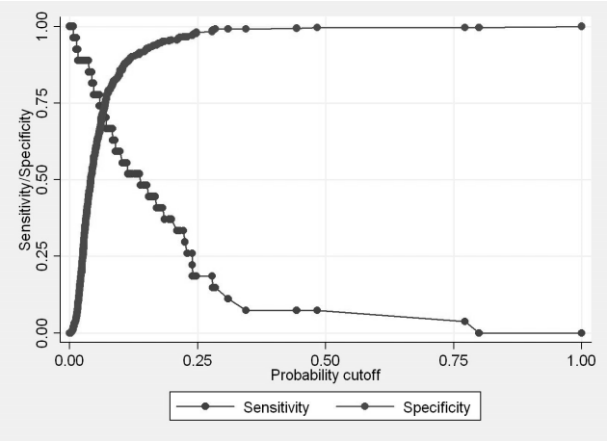
Recently progesterone therapy has been shown to markedly reduce preterm birth in women at high risk due to a previous preterm birth. Unfortunately there are no accurate predictors for women in their first pregnancy. We have previously shown that maternal plasma Corticotrophin Releasing Hormone (CRH) can predict preterm birth but with a relatively low sensitivity and specificity. We hypothesised that the endocrine pathways leading to uterine activation may respond to rate of change of endocrine signals rather than absolute levels and that rate of change of plasma CRH and other variables may predict preterm birth more effectively than absolute levels. Four hundred unselected women were prospectively followed to delivery with serial maternal blood samples taken at 4 weekly intervals. Samples were assayed for plasma CRH, human chorionic gonadotrophin (hCG) and other variables. Curves were fitted to time course data for each individual and each variable. Data at 26 weeks were then generated from the fitted functions. An exponential and a quadratic curve were used to model log transformed CRH and hCG respectively for 27 preterm and 364 singleton term pregnancies. Multiple logistic regression was performed to test the relationship between changes in the variables and the outcome of interest: preterm birth.



Results • At 26 weeks multivariate analysis revealed that a combination of Rate log CRH, log CRH and timing of HCG minimum with a probability cutoff of 0.18 produced a sensitivity of 40.74% and a specificity of 95.08% and correctly classified 91.35% of patients.

Conclusions. Like other endocrine systems it seems that gestational tissues respond to dynamic changes in regulatory processes rather than absolute concentrations. This can be exploited to more accurately predict events such as preterm birth. More accurate identification of obstetric risk may allow early intervention with preventative therapies such as progesterone supplementation.

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PROTON PUMP INHIBITORS CAUSE HYPOMAGNESAEMIC HYPOPARATHYROIDISM

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We report two cases of proton-pump inhibitor (PPI) induced hypomagnesaemic hypoparathyroidism. Both patients presented with carpopedal spasm and tetany in association with severe hypomagnesaemia and hypocalcaemia. Serum magnesium was 0.23 (Case 1) and 0.38 mmol/L (Case 2): (normal: 0.7-1.0 mmol/L); corrected calcium was 1.55 (Case 1) and 1.75 mmol/L (Case 2): (normal: 2.20-2.55 mmol/L); urinary magnesium was 0-1 mmol/day (normal: 2-8 mmol/day).

Calcium levels returned to normal with supplemental elemental calcium 2.4 g/day (Case 1) and calcium 2.4 g with 1,25(OH) Vitamin D 0.5 micrograms and magnesium aspartate 240mg elemental (Case 2). Magnesium homeostasis, however, could not be achieved despite up to 480 mg elemental magnesium supplements daily until proton pump inhibitors were withdrawn.

Following PPI withdrawal, magnesium homeostasis rapidly returned to normal in both blood and urine. Re-introduction of PPI therapy in case 1 rapidly induced hypomagnesaemia again.

Magnesium homeostasis remained normal after substitution of the PPI with ranitidine.

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SMALL CHANGES IN THYROXINE DOSAGE DO NOT PRODUCE MEASURABLE CHANGES IN HYPOTHYROID SYMPTOMS, WELL-BEING OR QUALITY OF LIFE: RESULTS OF A DOUBLE BLIND, RANDOMIZED CLINICAL TRIAL.

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Background: In patients with primary hypothyroidism, anecdotal evidence suggests that well-being is optimized by fine adjustment of thyroxine dosage, aiming for a serum TSH concentration in the lower reference range. This has not been tested in a clinical trial.

Objective: To test whether adjustment of thyroxine dosage aiming for a serum TSH concentration <2 mU/L improves well-being compared with a serum TSH concentration in the upper reference range.

Methods: Double blind, randomized clinical trial with a crossover design in 56 subjects (52 females) with primary hypothyroidism taking thyroxine ≥100 mcg/day) with baseline serum TSH 0.1-4.8 mU/L.

Interventions: Each subject received three thyroxine doses (low, middle, high; 25 mcg increments) in random order.

Outcome measures:Visual analog scales assessing well-being (the primary endpoint) and hypothyroid symptoms, quality of life instruments (General Health Questionnaire 28, SF-36 and Thyroid Symptom Questionnaire), cognitive function tests, treatment preference and clinical and biochemical markers of thyroid hormone action.

Results: Mean (± SEM) serum TSH concentrations were 2.8 ± 0.4, 1.0 ± 0.2 and 0.3 ± 0.1 mU/L for the three treatments. Significant treatment effects were observed on the following markers of thyroid hormone action: ankle jerk relaxation time, serum SHBG, plasma cholesterol and urine deoxypyridinoline/creatinine ratio ($P<0.001$ in each case). There were, however, no significant treatment effects on any of the instruments assessing well-being, symptoms, quality of life or cognitive function, and no significant treatment preference.

Conclusions: Small changes in thyroxine dosage do not produce measurable changes in hypothyroid symptoms, well-being or quality of life, despite the expected changes in serum TSH and markers of thyroid hormone action. These data do not support the suggestion that the target TSH range for the treatment of primary hypothyroidism should differ from the general laboratory range.

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PAMIDRONATE OR ZOLEDRONIC ACID REDUCE BONE LOSS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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Rapid and early bone loss occurs after allogeneic stem cell transplantation (alloSCT). We evaluated the effects of high-dose pamidronate therapy on bone mineral density (BMD) after alloSCT in a randomised, multicentre open-label 12-month prospective study of 116 patients who received intravenous pamidronate (90 mg/month) beginning just prior to conditioning versus no pamidronate. All patients also received calcitriol (0.25 µg/day) and calcium (1000 mg/day), which were both continued for a further 12 months. The primary study outcome compared changes in BMD, measured by dual-energy x-ray absorptiometry (DXA), at 12 months post-alloSCT at the femoral neck, lumbar spine and total hip between the treatment arms, and the influence of glucocorticoid and cyclosporin therapy on these changes. Compared with the no pamidronate group, pamidronate reduced bone loss at the spine, femoral neck and total hip by 5.6%, 7.7% and 4.9% (all $p<0.003$), respectively, at 12 months. However, BMD of the femoral neck and total hip was still 2.8% and 3.5% lower than baseline, respectively, ($p<0.05$) in the pamidronate group. At 24 months, only differences at the total hip remained significant higher in the pamidronate group by 3.9% ($p<0.05$). Benefits of pamidronate therapy on BMD were restricted to patients receiving an average daily prednisolone dose >10 mg and prolonged cyclosporin therapy within the first six months of alloSCT. In a subsequent uncontrolled, prospective study of 12 patients receiving zoledronic acid (ZA), within the first year post-alloSCT, BMD was measured pre-transplant, pre-ZA and post-ZA. The median annualised percentage change in femoral neck (FN) BMD pre-ZA was –13.2% (range –40% - +1.0%). Post-ZA, FN-BMD increased by a median of +1.4% (range –22.2% - +33.6%). One patient continued to lose bone from the FN post-ZA infusion. In conclusion, pamidronate markedly reduced bone loss after alloBMT, but did not completely prevent it at the femoral neck and total hip. Benefits on BMD were greatest in patients on higher doses of immunosuppressive therapy, but most were lost 12 months after stopping pamidronate. ZA may be more effective at

preventing bone loss after alloSCT. This now requires confirmation in a larger prospective, randomised dose-finding study.

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MULTIFOCAL PAPILLARY THYROID CARCINOMA ARISING IN HASHIMOTO'S THYROIDITIS

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A 45 year old woman requested assessment of her thyroid status after learning that her sister in Bosnia had been diagnosed with an "overactive thyroid". She had no significant past history apart from time spent in Bosnia which was at the time of the Chernobyl nuclear disaster. She had no symptoms to suggest thyroid dysfunction, and examination was unremarkable. Her TSH was mildly elevated at 6.4mU/L (NR 0.3 – 5.0) with a normal fT4 of 10.3pmol/L (7.5 – 21). A thyroid ultrasound and technetium uptake study revealed a cold nodule in the inferior pole of the right lobe; a fine needle aspirate revealed cells consistent with a papillary carcinoma. A pre-operative CT scan revealed extensive lymphadenopathy in the upper mediastinum and right side of the neck. A total thyroidectomy and lymph node clearance was carried out, complicated only by temporary hypoparathyroidism. Histopathology demonstrated multifocal thyroid papillary carcinoma arising in the setting of extensive Hashimoto's thyroiditis with only reactive changes in all thirteen lymph nodes. She has subsequently received conventional ablative radioiodine therapy with a plan for ongoing surveillance.

Lymphoma is the malignancy most often reported as being associated with Hashimoto's thyroiditis. [1] There are however reports of an increased incidence of papillary thyroid carcinoma within Hashimoto's thyroiditis [2] and emerging evidence suggesting an overlap in their morphological, immunohistochemical and molecular features. [3] [4] [5] In particular, the RET/PTC rearrangement, which is considered a specific marker for papillary thyroid carcinoma, is also found in Hashimoto's thyroiditis. [6] [7] Patients exposed to the Chernobyl radioactive fallout develop not only RET/PTC driven papillary cancer but also chronic autoimmune thyroiditis. [8] [9]

This case highlights that although Hashimoto's thyroiditis is considered a benign condition, there is a strong link with papillary thyroid carcinoma. Clinicians need to be aware of this connection and investigate nodular changes within Hashimoto's thyroiditis accordingly, although it is too early to rely on molecular markers to predict which patients will develop malignancy.

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HYPERSEROTONINAEMIA IN GILBERT'S SYNDROME MIMICKING CARCINOID SYNDROME -- A NOVEL MECHANISM?

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The involvement of serotonin in carcinoid syndrome and various neuropsychiatric disorders has attracted extensive research in recent decades. Hyperserotoninaemia associated with non-specific gastrointestinal symptoms has led to the suggestion of an ill-defined clinical entity sometimes known as Serotonin Irritable Syndrome. We report a case of hyperserotoninaemia in the absence of a detectable carcinoid tumour in a 27 year old woman with Gilbert's Syndrome who presented with 10 months history of night sweats, palpitations, facial flushing and intermittent watery diarrhoea. Biochemistry revealed normal urinary 5-hydroxyindoleacetic acid (HIAA) excretion, but persistently elevated serum serotonin up to 2456 nmol/L (reference interval: 0-1200 nmol/L), with elevated urinary serotonin excretion during one

exacerbation and elevated platelet serotonin on one occasion. No mass was found on CT of the chest and abdomen, and no increased uptake was seen on an octreotide scan.

Patients with Gilbert's syndrome are known to be deficient in glucuronidation of bilirubin due to genetic defects in UDP-glucuronosyltranserase (UGT) 1A1, leading to hyperbilirubinaemia. Genetic polymorphism of UGT1A6 results in altered glucuronidation of serotonin, leading to impaired renal elimination. Co-occurrence of UGT1A1*28 and UGT1A6*2 has been described although its clinical significance is uncertain. Such dual genetic polymorphism could result in altered serotonin metabolism with increased uptake into platelets[1] and decreased clearance. We have confirmed homozygocity for both UGT1A1*28 and UGT1A6*2 polymorphisms in our patient, giving a possible cause for the hyperserotoninaemic state.

Gilbert's Syndrome is thought to be a benign disorder with no impact on long term mortality. This case however illustrates a possible mechanism of the frequently reported non-specific symptoms including fatigue, abdominal pain, diarrhoea, and sweating in Gilbert's Syndrome, mimicking Carcinoid Syndrome.

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SUBEROYLANILIDE HYDROXAMIC ACID (SAHA), A HISTONE DEACETYLASE INHIBITOR, REPRESSES ANDROGEN RECEPTOR EXPRESSION AND ACTS SYNERGISTICALLY WITH AN ANDROGEN RECEPTOR ANTAGONIST TO INHIBIT PROSTATE CANCER CELL PROLIFERATION

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Growth of prostate cancer cells is initially dependent upon androgens, and androgen ablation therapy (AAT) is used to control tumour growth. Unfortunately, resistance to AAT inevitably occurs, and this stage of disease is resistant to conventional chemotherapeutics. Consequently there is an urgent need for better strategies to treat advanced prostate cancer. Histone deacetylase inhibitors, such as suberoylanilide hydroxamic acid (SAHA), are promising agents for a range of malignancies, including prostate cancer. Here we have demonstrated that SAHA inhibits proliferation of the androgen responsive prostate cancer cell line, LNCaP, at low micromolar concentrations (< 5 μM), causing cell cycle arrest and induction of $p21^{WAF1}$ expression. Treatment with SAHA (> 5μM) induced caspase-dependent apoptosis of LNCaP cells. Gene profiling and immunoblot analysis demonstrated a decrease in androgen receptor (AR) mRNA and protein in LNCaP cells cultured with SAHA compared to control cells, with a corresponding decrease in levels of the AR-regulated gene, prostate specific antigen. Culture of LNCaP cells in steroid-free medium markedly sensitised the cells to SAHA and, moreover, combining low concentrations of SAHA and the AR antagonist bicalutamide, that individually have no effect on cell growth, resulted in a synergistic reduction in cell proliferation and increase in caspase-dependent cell death (synergy confirmed by isobole statistical analysis). At these low concentrations, SAHA and bicalutamide had no effect, alone or in combination, on proliferation or death of AR-negative PC-3 prostate cancer cells. Taken together, our findings suggest that SAHA is effective in targeting AR signalling, and that androgen withdrawal or blockade may sensitise prostate cancer cells to cell death induced by histone deacetylase inhibitors. Given that the majority of clinical prostate tumours express AR, including those that fail hormonal therapy, further investigation into the use of SAHA for treatment of prostate cancer is warranted, particularly in the context of combination therapy with conventional AAT.

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EXPRESSION OF THE ANDROGEN RECEPTOR AND ITS ASSOCIATION WITH DISEASE OUTCOME IN BREAST CANCER

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The estrogen receptor (ER) and progesterone receptor (PR) have been extensively studied in breast cancer and associated with tumour grade, treatment response and disease outcome. In contrast, the role of the androgen receptor (AR) in breast cancer is less well understood. In this study we investigated the relationship between the expression of AR and disease outcome by immunohistochemical staining in a cohort of 194 invasive breast cancers. Nuclear AR staining was measured by visual assessment and manual counting. Positive AR immunostaining ($\geq$ 10% positive cells) was observed in 73% of tumours and was inversely related to tumour size (χ^2; p value = 0.0053), histological grade (χ^2; p value = 0.0001), and was significantly associated with ER (χ^2; p value <0.0001) and PR (χ^2; p value <0.0001). Negative AR immunostaining (<10% positive cells) was associated with a 2.7 fold (*P*=0.011) increased risk of relapse and a 4.8 fold (*P*=0.0002) increased risk of cancer-related death. The relationship between negative AR immunoreactivity and poor outcome was observed in node positive breast cancer but not node negative disease. In node positive patients, only tumour grade and AR status were predictors of disease-relapse. These findings are consistent with our recent studies indicating that the AR signalling pathway is a determinant of breast cancer growth, potentially via an effect on ER signalling.

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OLDER MEN WITH ORGANIC ANDROGEN DEFICIENCY (AD) MAINTAIN SIMILAR TROUGH AND PEAK BLOOD TESTOSTERONE LEVELS AND QUALITY OF LIFE AS YOUNGER AD MEN WITHOUT CHANGE IN TESTOSTERONE IMPLANT DOSE.

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Classical AD due to organic disorders of hypothalamus-pituitary or testes requires life-long testosterone replacement. Yet whether testosterone dosage should be modified at older age has not been studied. We reviewed retrospective data from men with classical AD over 65 yr (OAD, n=24, 71±1 yr) compared with younger (<55yr) AD men (YAD, n=25, 40±3 yr) receiving long-term testosterone implants (800 mg at 5-6 month intervals) with groups well matched for type of hypogonadism, height, weight and BMI. Both trough (10.7±0.8 nM vs 10.3 ± 1.4 nM) and 1 month peak (22.1±0.9 vs 24.4±1.1 nM) blood testosterone concentrations were similar in OAD and YAD men, respectively. In men with primary hypogonadism, FSH was significantly (47.3±4.6 vs 26.4±6.8 IU/L p=0.01) and LH non-significantly (25.5±3.3 vs 18.9±4.6 IU/L p=0.24) higher in YAD men. Although blood PSA was significantly higher in OAD men (1.7±0.6 vs 0.9±0.1 μg/L, p<0.005) all values remained within the adult reference range. On the SF-36 scale, compared with age-matched Australian norms, OAD men had significantly worse scores for bodily pain, vitality and mental health whereas YAD men had significantly worse physical role limits, general health, vitality, social functioning and mental health. OAD men had no more self-reported co-morbidities (hypertension, ischaemic heart disease, cerebrovascular accident, osteoporosis, osteoarthritis, diabetes) than the age-matched Australian population. Our findings suggest that metabolism of exogenous testosterone is unaffected but hypothalamic androgenic negative feedback sensitivity is reduced with older age. Quality of Life for OAD remains at least as good as the YAD compared with age-matched Australian norms and the older men have no more co-morbidities than age-matched Australian controls. These findings provide no basis to modify testosterone dosage in men with classical AD at older age. However, the validity of extrapolation beyond this OAD cohort especially to older men with only age-related decline in blood testosterone is unclear.

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MECHANISMS UNDERLYING INHIBITION OF ANDROGEN-INSENSITIVE PROSTATE CANCER CELL PROLIFERATION BY PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA.

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Current treatments for prostate cancer are potentially curative for patients with localized disease or early stage tumors, but only palliative in patients suffering metastatic disease. Innovative therapeutic approaches are required to reduce treatment-related morbidity and improve outcomes. Recent studies have shown aberrant activation of the Wnt signal transduction pathway is involved in the development of prostate cancer. Activation of Wnt signaling disrupts the glycogen synthase kinase 3-β (GSK3-β) complex allowing accumulation and translocation of β-catenin into the nucleus to stimulate cell proliferation. Peroxisome proliferator-activated receptor gamma (PPARγ) modulates cell proliferation and cell cycle progression but the interaction between PPARγ and Wnt signaling in prostate cancer is not well understood. We assessed the effects of the PPARγ agonists ciglitazone (thiazolidinedione) and indomethacin (non-steroidal anti-inflammatory drug), to mediate suppression of prostate cancer cell growth and characterized the underlying mechanisms using DU145 cells as a model of androgen independent prostate cancer. Ciglitazone and indomethacin significantly enhanced PPARγ activity by 1.6-fold ($P<0.05$) and 1.94-fold ($P<0.05$) respectively and slowed DU145 cell proliferation by 22% (Cig, $P<0.01$) and 20% (Indo, $P<0.05$). The effect of these ligands was not achieved through regulation of PPARγ mRNA and protein expression. TUNEL assays showed that ciglitazone significantly induced a 17-fold increase in apoptosis ($P<0.01$), compared to control, suggesting that apoptosis may be an alternate mechanism by which ciglitazone slows DU145 cell proliferation. Western analysis indicated that ciglitazone and indomethacin significantly decreased β-catenin protein levels in DU145 cells consistent with an effect of these ligands to inhibit Wnt signaling. In conclusion, ciglitazone and indomethacin inhibit proliferation of androgen-insensitive DU145 human prostate cancer cells with a differential effect on apoptosis. Inhibition of Wnt signaling by PPARγ agonists may contribute to their effects on DU145 cell proliferation. Further exploration of the PPARγ -Wnt interaction may help clarify newer therapeutic strategies for androgen-insensitive prostate cancer.

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DEVELOPMENT OF DOMINANT NEGATIVE ANDROGEN RECEPTORS AS A NOVEL THERAPEUTIC STRATEGY FOR PROSTATE CANCER

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There is increasing evidence that over-expression and/or aberrant expression of the androgen receptor (AR) contributes to failure of hormonal therapies for prostate cancer and that directly targeting the receptor may be a more effective way of inhibiting the growth of these tumours. The AR is a member of the steroid receptor superfamily that shares similar structural and functional domains including the highly conserved DNA binding domain (DBD) and ligand binding domain (LBD), the latter of which contains the activation function, AF-2. The transcriptional activity of the AR occurs predominantly through the amino-terminal transactivation domain (NTD) of the receptor through two activation functions, AF-1 and AF-5. This study shows that deletion of a region of the NTD (amino acid 38-410), including AF-1 and part of AF-5, results in a dominant negative AR (DNAR) that inhibits both wild type and mutant AR in prostate cancer cells by up to 95%. Upon further investigation, we demonstrated a specific interaction between the DNAR and wtAR, suggesting that the DNAR may inhibit wtAR activity via receptor dimerisation. Subsequently, we examined the requirements for dimerisation through each receptor domain. Our results suggest that activity of the DNAR does not require an N/C-terminal interaction which is characteristic of agonist induced wtAR function, and occurs through binding of AF-2 and a peptide (^{23}FXXLF^{27}) in AF-1. Deletion and mutational studies demonstrated than an intact DNA binding domain and dimerisation through the ligand binding domain are essential for optimal DNAR activity. These studies provide a basis for developing novel AR-targeted therapies for prostate cancer, as well as generating new insights into AR structure and function.

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GLOBAL AND MUSCLE-SPECIFIC ANDROGEN RECEPTOR KNOCKOUT MICE DEMONSTRATE DIRECT ANABOLIC ACTIONS OF ANDROGENS IN SKELETAL MUSCLE

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We have generated global and muscle-specific androgen receptor (AR) knockout (ARKO) mice, to dissect the molecular mechanisms of androgen action in skeletal muscle. ARKO mice have exon 3 of the AR gene targeted, leading to deletion of the 2nd zinc finger of the DNA binding domain. We previously showed a 15-20% decrease in hindlimb muscle mass in male global ARKOs, and complete failure of the androgen sensitive levator ani (LA) muscle to develop. Two muscle-specific ARKO (mARKO) lines, generated using either α-actin-cre or MCK-cre, also have decreased muscle mass, with mass reduced by 8-14% in hindlimb muscles of mARKO males, and by 50% in the LA muscle, associated with a significant reduction in fibre cross-sectional area. We performed microarray analysis on RNA from control and global ARKO male muscles (n=2/grp), using the Affymetrix mouse array 430. Genes with >1.5 fold-change in duplicates were identified, and gene ontology mining performed to identify androgen-responsive functions. A suite of ~120 genes shows differential expression in muscle of ARKO mice. There is a significant increase in the expression of genes encoding muscle contractile proteins characteristic of slow-twitch phenotype. This fast-to-slow switch is consistent with our previous *in vitro* physiology findings, which demonstrated a decrease in force and increase in fatigue resistance in ARKO muscles. There is also decreased expression of the polyamine biosynthetic enzymes adenosyl-methionine decarboxylase (AdoMetDC) (4.8-fold decrease, $p<0.001$) and ornithine decarboxylase (ODC) (2.5-fold decrease, $p<0.001$). Polyamine actions have been implicated in numerous cellular functions [1], and we hypothesise they may partly mediate the anabolic actions of androgens in muscle. We are currently confirming gene expression using quantitative real-time PCR. These data demonstrate that androgens act directly through the AR in muscle to regulate muscle mass and function, and shed light on the molecular pathways mediating androgen actions in muscle.

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LOW TESTOSTERONE LEVELS ARE NOT COMMON IN MEN WITH TYPE 2 DIABETES MELLITUS AND, IN CONTRAST TO LOW SHBG LEVELS, NOT ASSOCIATED WITH POOR GLYCAEMIC CONTROL.

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Background: Recent studies have found a high frequency (33%) of low testosterone (T) levels in men with type 2 diabetes mellitus (T2DM)[1], and low T levels have been shown to be associated with poor glycaemic control [2].

Methods: We determined total testosterone (TT, ACCESS Immunoassay, Beckman Coulter, manufacturers reference range of 6.1-27.1 nmol/L validated at Austin in 240 healthy males, mean age 41 years), SHBG, calculated free T (cFT [3]), HbA1c, and fasting blood glucose in 167 consecutive men with T2DM attending our diabetes outpatient clinics. In the subgroup not treated with insulin, we measured insulin levels and calculated the HOMA-IR index [4]. Student's t test was used to compare the means between quartiles.

Results: Mean age was 68.1+0.85 years (range 32-87), BMI 29.6+0.4 kg/m2 (19-45), HbA1c 7.6+0.09% (5.3-12.7) TT 11.7+0.33 nmol/L (1.0-24.2), SHBG 36.2+1.3 nmol/L (7-88), cFT 0.239+0.007 nmol/L (0.019-0.527), and duration of T2DM 13.2+0.7 years (0.1-49.4). 12 men (7.2%) had low TT levels (<6.1 nmol/L), 13 (7.7%) had low cFT levels (<0.119 nmol/L), and 17 (10.1%) had either a low TT or a low cFT level. Men with TT levels in the lowest quartile had similar HbA1c levels than men with TT levels in the highest quartile, 7.53+0.15% vs 7.41+0.15% ($p=0.59$), and the same was found for cFT quartiles, 7.47+0.2% vs 7.68+0.1% ($p=0.25$). Compared to men with SHBG levels in the highest quartile, men with SHBG levels in the lowest quartile were younger, 60.7+1.9 vs 73.1+1.3 years ($p<0.001$), more obese, BMI 31+1.9 vs 27.7+0.76 kg/m2 ($p=0.004$), had lower TT levels, 9.65+0.2 vs 14.0+0.2 nmol/L ($p<0.001$), higher HbA1c levels, 7.97+0.23 vs 7.51+0.14 % ($p=0.07$), slightly higher insulin levels, 75.5+7.8 vs 67.1+10.9 pmol/L ($p=0.52$), and were slightly more insulin resistant, HOMA-IR 1.63+0.17 vs 1.36+0.21 ($p=0.33$).

Conclusions: In contrast to previous studies, prevalence of low T levels in our cohort of men with T2DM was not higher than expected for age-matched non-diabetic men [5]. Low SHBG levels were better predictors of glycaemic control than low T levels, presumably because SHBG levels correlate inversely with body weight and insulin resistance.

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THE GROWTH HORMONE RECEPTOR IS CONSTITUTIVELY DIMERIZED AND ACTIVATED BY ROTATION OF THE CYTOPLASMIC DOMAINS

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Growth Hormone (GH) is the major regulator of postnatal growth, an important regulator of adult metabolism and is implicated in a number of disease states. The human GH Receptor (hGHR) is believed to be activated by the binding of GH sequentially to two monomeric receptors and this dimerization allows intracellular signalling. However, our group recently determined that the hGHR exists as constitutive dimers in living cells, by using Fluorescence Resonance Energy Transfer (FRET)[1]. FRET also showed that the extent of dimerization is unaffected by GH. Furthermore, we were able to demonstrate that the closely related Prolactin Receptors also exist as constitutive dimers in vivo. By truncating the intracellular and extracellular domains (ICD and ECD) of the hGHR, it was shown that the transmembrane domain (TMD) is required for stabilizing constitutive hGHR dimerization. The ToxR System, a bacterial assay for examining TMD interactions, was then used and it was found that constructs consisting of various parts of the hGHR TMD produced robust beta-galactosidase signal, indicating interaction along the length of the TMD. Finally, to determine the actual activation mechanism of the constitutively dimerized GHR, sequential alanine residues were inserted to rotate the ICD at the lower TMD boundary. Only one insertion in could make the full length hGHR activate the intracellular signalling molecules of JAK2 and STAT5 in the absence of GH. Taken together, these results support a model for hGHR activation by rotation of preassociated receptors, rather than by hormone induced dimerization of two monomers. This new mechanism for hGHR activation will facilitate the future rational design of GH mimetics for use in cancer treatments and tissue regeneration.

This work was supported by NHMRC Australia.

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REMOVAL OF BOX1 FROM THE GH RECEPTOR ABOLISHES JAK/STAT SIGNALLING BUT NOT MAPK

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The growth hormone receptor (GHR) is responsible for regulation of post-natal growth in mammals and is involved in many endocrine and metabolic pathways. The GHR, a member of the class 1 cytokine superfamily, contains a proline rich region termed box 1, which binds Jak2 and facilitates signalling to pathways such STAT5 and MAPK. We have previously targeted the cytoplasmic domain of the GHR in vivo, through these truncations we have determined that STAT5 activation was completely abolished with removal of the 8 distal tyrosines but 30% remained after removal of the distant 5. In this study we have converted the 4 conserved prolines in the Box1 region to alanine, to observe the in vivo effects of removing Jak2 mediated growth hormone (GH) signalling (Box1 mutant). Growth parameters were measured and compared with both of our mutants; wildtype and the GHR null mice. Homozygous Box1 mutants showed similar growth retardation (45%) to WT as the mutant 391 (49%) and the GHR null mice (42%). With pre-pubescent GH stimulation Jak2 activation was absent as was the associated STAT5 activation, however MAPK activation was found to be present dissociating this pathway from the requirement of Jak2 activation. IGF-1 levels were lowered to <10% and IGFBP-3 was absent in serum. Bone lengths were reduced as a result and brain size was disproportional with growth as reported for other models. STAT5 dependent major urinary protein was absent in Box1 mutants also. Microarray analysis and Quantitative Real time PCR has revealed some transcripts that are differentially expressed between the phenotypically very similar Box1 and the GHR null mice, which highlights transcripts that are under the control of remaining active pathways. Further phenotypic analysis is currently being undertaken especially into the pathway by which the GHR activates MAPK pathway. This work was supported by NHMRC (Australia).

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CONTRASTING REGULATORY EFFECTS OF SELECTIVE OESTROGEN RECEPTOR MODULATORS ON GH SIGNALLING IN BREAST AND KIDNEY TISSUES

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GH is an important regulator of body metabolism, with action regulated by oestrogen and the selective oestrogen receptor modulators (SERMs)[1]. GH action is mediated by the JAK2/STAT5 pathway, which is terminated by two classes of inhibitors: the suppressors of cytokine signalling (SOCSs) and protein tyrosine phosphatases, SHP-1 and SHP-2. We have recently reported that oestrogen inhibits GH signalling by stimulating SOCS-2 expression[2]. In contrast, SERMs exert enhancing effects by reducing SHP activities. While these observations were made in kidney (HEK293) cells, the action of SERMs in other tissues is not known. In this study, we investigated the effects of SERMs on GH signalling and the inhibitors in breast tissue, an important target of actions of GH and oestrogen. In breast cancer (MDA-MB-231) cells expressing human GH receptor and estrogen receptor-α, 17β-oestradiol (E_2) reduced GH activation of a STAT5-responsive luciferase reporter and JAK2 phosphorylation to 60±5% and 45±6% of GH-treated control (mean±SE; $P<0.05$), respectively. 4-Hydroxytamoxifen and raloxifene did not affect GH action, a finding very different to that in kidney cells. Instead, the two SERMs effectively attenuated the inhibitory effects of E_2, an action similar to the anti-oestrogen ICI182780. E_2 increased the SOCS-2 mRNA level by 139±3% of untreated control ($P<0.001$), while SERMs had no effect. SHP activities were not altered by E_2 or SERMs. In summary, oestrogen inhibited GH signalling and stimulated SOCS-2 expression in breast cancer cells. SERMs did not affect GH signalling and the inhibitors in these cells, but antagonised the inhibitory effect of oestrogen. We conclude that SERMs modulate GH signalling in a cell-type dependent manner. The concurrent lack of effects on GH signalling and SHPs supports a functional role of phosphatases in the enhancement of GH action by SERMs. (Supported by NHMRC)

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REDUCED IGFBP-3 ABUNDANCE CONTRIBUTES TO CATCH-UP GROWTH IN THE INTRAUTERINE GROWTH-RESTRICTED LAMB.

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Most infants who are small at birth undergo accelerated, or catch-up growth, in early postnatal life. This catch-up growth predicts adult height, and is also an independent risk factor for metabolic and cardiovascular disease, including diabetes, obesity and hypertension. Poor placental growth or function is a major cause of low birth weight in humans, and we have shown that experimental restriction of placental growth (PR) in sheep (1) is followed by catch-up growth in neonatal lambs. We have previously reported that circulating abundance of the anabolic hormones, insulin-like growth factor (IGF)-I and IGF-II is unaltered in the PR lamb, but that total IGF-binding capacity of plasma is reduced, suggesting increased IGF bioavailability (2). We therefore investigated the effects of PR on abundance in plasma of individual IGF-binding proteins (IGFBP) in the young lamb, and their relationships with catch-up growth.

Plasma samples were collected, and weight and size measured at 5-day intervals during catch-up growth in control (n=14) and PR lambs (n=12). Abundances of IGFBP at 5 and 10 days of age were quantitated by Western blotting using ^{125}I-IGF-II as ligand.

PR tended to decrease plasma IGFBP-3 at 10 days (9% lower, $P=0.081$), although not at 5 days of age. PR did not alter the plasma IGFBP-2 at 5 or 10 days of age. Plasma IGFBP-3 at 10 days of age correlated negatively with neonatal growth rates of long bones. Plasma IGFBP-2 at 5 and 10 days of age correlated negatively with neonatal growth rates of abdominal circumference.

These data support our hypothesis that decreased IGFBP abundance, particularly of IGFBP-3, contributes to neonatal growth by increasing the bioavailability of IGFs.

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INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN-2 IS AN ESSENTIAL REGULATOR OF NEUROBLASTOMA CELL MOTILITY

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IGF binding proteins (IGFBPs) regulate IGF bio-availability and signalling. Increasing evidence suggests that some IGFBPs modulate cell survival, proliferation and migration independent of IGFs. A key event is the interaction of IGFBP with cell membrane "receptors", but mechanisms involved remain unclear. IGFBP-2 interacts with cell membrane proteoglycans and integrin via its heparin binding (HBD) or integrin binding (RGD) motifs. We have demonstrated, via an HBD mutant IGFBP-2, that the HBD of IGFBP-2 is required for enhancement of proliferation, migration and invasion of neuroblastoma (NB) cells. Whether IGFBP-2 acts via regulation of cell adhesion and motility genes is unknown.

We therefore performed real-time PCR on mRNAs extracted from SHEP cells over-expressing WT or HBD mutant IGFBP-2 and quantified expression of genes involved in cell motility.

A range of genes was *up-regulated* by WT-IGFBP-2, including those promoting cell migration and invasion (>2fold): MMP2/13 (matrix-metallo proteases), MTA1 (metastasis associated 1), c-myc and VEGF The same genes were down-regulated (>2fold) by over-expression of the non-binding HBD-IGFBP-2 mutant.

Over-expression of WT-IGFBP-2 also *down-regulated* (>2fold) genes whose loss of expression is strongly associated with increased cell motility in cancer cells: CDH1 (E-cadherin), CDH6 (K-cadherin), EPHB2 (ephrin B2). The same genes were *up-regulated* (>2fold) in the HBD-IGFBP-2 over-expressing SHEP cells.

WT-IGFBP-2 over-expression markedly increased levels of aVb3 integrin, known to be associated with NB cell aggressiveness. Levels were low in the HBD-IGFBP-2 over-expressing mutant.

IGFBP-2 induced migration and invasion of NB cells is thus associated with altered expression of genes favouring those supporting these processes, an effect clearly abrogated by mutation of the HBD motif. Our data suggest that high expression of IGFBP-2 in NB cells, as in other cancer cells, is a major determinant of adhesion, motility and metastasis. These processes, requiring IGFBP-2 attachment to cell surface, are mediated via specific gene regulation.

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GH REGULATION OF METABOLIC GENES IN MUSCLE: A MICROARRAY STUDY IN HYPOPITUITARY MEN

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GH regulates substrate metabolism in muscle and liver, both of which are major contributors to the whole body metabolic process. Little is known about the effects of GH on metabolic gene expression in muscle. The aim of this study is to identify GH-responsive genes in skeletal muscle that regulate substrate metabolism. Six hypopituitary men underwent substrate metabolism measurement with muscle biopsies collected before and after two weeks of GH treatment (0.5mg/day). Serum IGF-I, procollagens I and III were measured by RIA. Gene expression profiles of four subjects were analysed using the Affymetrix GeneChips. Selected responsive genes were verified by quantitative RT-PCR. GH increased serum IGF-I, procollagens I and III, enhanced lipid oxidation, reduced carbohydrate oxidation, and stimulated protein synthesis. GH induced differential expression of 217 genes in muscle. It stimulated gene expression of IGF-I and collagens, including COL1A1, COL1A2 and COL3A1. It reduced expression of several enzymes regulating lipid uptake and oxidation, and that of glycogen synthase 1 and pyruvate dehydrogenase kinase 4, suggesting a shift to glucose utilization for energy production. GH reduced expression of proteolytic genes, and exerted a mixed effect on those in protein synthesis. Interestingly, GH increased expression of circadian gene CLOCK, and reduced that of PERIOD 1 and CRYPTOCHROME 2. The responses of IGF-I, COL3A1 and CLOCK were confirmed by RT-PCR. In conclusion, GH stimulates expression of muscle IGF-I and collagens that may contribute to the increased levels in blood. It also regulates expression of a range of genes involved in lipid, carbohydrate and protein metabolism. The discordance between muscle gene expression profiles and the whole body metabolic responses suggests that muscle is unlikely to contribute to the GH-stimulated lipid oxidation. GH may regulate diurnal function in muscle, providing a connection between metabolism and circadian rhythmicity. (Supported by NHMRC)

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THE INFLUENCE OF DEMOGRAPHIC FACTORS ON THE RATIO OF 20-kDA AND 22-kDa GH ISOFORMS AND THE UTILITY OF THE RATIO FOR DETECTION OF GH DOPING IN SPORT.

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Measurement of the 20-kDa (20K) and 22-kDa (22K) isoforms of GH can be used to detect GH doping, since administration of exogenous 22K GH changes the 22K/20K isoform ratio. To determine the influence of demographic factors and sporting type, the isoforms were measured in serum samples from 972 elite athletes (M=606, F=366), representing 4 major ethnic groups. The 20K and 22K GH isoforms were measured by specific ELISAs with the detection limit being 10 pg/ml for each assay. Multiple regression analysis was used to estimate the effects of age, gender, BMI, ethnicity and sporting type. Detectable measurements were obtained for 20K and 22K GH in 87% and 98% of samples respectively. The 20K and 22K GH values were not normally distributed, with median and inter-quartile ranges (IQ), pg/ml: 20K 74, 30-242; 22K 420, 187-1466. Both isoforms significantly decreased with age, were significantly higher ($P<0.0001$) in women (mean ± SE, pg/ml: 20K 297 ± 20; 22K 2195 ± 108) than in men (20K 176 ± 11; 22K 1001 ± 66) and were negatively correlated with BMI. The median 22K/20K ratio was 7.9 (IQ: 5.5 - 11.0) and was not significantly different between women and men. The 22K/20K ratio was normally distributed after log transformation. Multiple regression analysis indicated minimal contributions to between-subject variability of the log 22K/20K ratio from age (0.3%), gender (1.98%), BMI (0.02%), ethnicity (3.5%) and sporting type (1.3%); in total explaining 7.1 % of the log ratio variability. In elite athletes, the log 22K/20K ratio is minimally influenced by demographic factors and sporting type. The stability of the ratio to the effects of these factors renders it a promising measure of exogenous 22K GH abuse and this is being evaluated currently by our group. Supported by the World Anti-Doping Agency and Australian Government Anti-Doping Research Program.

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MODULATORY EFFECT OF RALOXIFENE AND OESTROGEN ON THE METABOLIC ACTION OF GH IN HYPOPITUITARY WOMEN

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We have previously reported that raloxifene (R) induces a lesser IGF-I suppressive effect than oestrogen in normal and GHD women. To investigate the GH-modulatory effects of these oestrogen compounds, we compared the impact of R and 17ß-oestradiol (E2) in GHD women during GH treatment. 17 hypopituitary women were randomised in a cross-over study to 1 month treatment with GH alone (0.5 mg/d) or in combination with R (60 mg/d) or E2 (2 mg/d) with a 2-4 wk washout. Endpoints were IGF-I and IGFBP-3, fat oxidation (Fox, quantified by indirect calorimetry), and whole body leucine turnover technique from which leucine turnover and incorporation into protein (LIP) were estimated. Results are expressed as change Δ ± SEM from baseline.

GH significantly stimulated all outcome measures. Cotreatment with E2 and R significantly reduced IGF-I while only E2 lowered IGFBP-3. Cotreatment with R but not E2 reduced Fox. The stimulation of LIP by GH was unaffected by E2 but reduced by R to a level that approached statistical significance.

Outcome Measure	GH	GH + R	GH + E2
Δ IGF-I nmol/l	18.9 ± 2.8*	11.4 ± 2.1*^	7.7 ± 2.1*^
Δ IGFBP-3 µg/ml	1.13 ± 0.1*	1.27 ± 0.2	0.18 ± 0.2^#
Δ Fox mg/min	14.2 ± 4.6*	2.1 ± 5.2^	8.1 ± 6
Δ LIP µM/min	17.6 ± 4.6*	8.9 ± 2.5†	18.9 ± 9.5

$p<0.05$ * vs. baseline, ^ vs. GH, # vs. GH+R; † $p=0.08$ vs. GH

In summary, during GH replacement of GHD women, E2 but not R reduced IGFBP-3 while R but not E2 antagonized fat and protein metabolism. We conclude, that in the doses used, R exerts a greater inhibitory effect than E2 on the metabolic action of GH. The mechanism is unknown but may involve IGFBP-3 mediation. (Supported by the NHMRC and by Lilly Australia.)

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MICROARRAY ANALYSIS OF GROWTH HORMONE RESPONSIVE GENES IN PERIPHERAL BLOOD LEUKOCYTES IN VIVO

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There is strong evidence that growth hormone (GH) activates the immune system both directly, and indirectly through IGF-1 and the cytokine network. Little is known, about what genes are regulated by GH in immune cells in vivo. The aim of this study is to investigate the effects of GH on gene expression in leukocytes.

Healthy male subjects, recruited as part of a intervention study aimed at developing a GH doping test, were administered 2mg/day GH for eight weeks followed by a 6 week washout period. Total RNA was extracted from white blood cells collected at baseline (week 0), weeks 4 and 8 (GH treatment) and week 14 (GH washout). Gene expression analysis was performed using Affymetrix HG-133 Plus 2.0 human genome arrays, which consist of 54925 probe sets, and the data analysed by GeneSpring software. Differential expression was analysed by one-way ANOVA.

In preliminary analysis of data from 4 subjects, GH induced significant change in 1049, 1463 and 690 probe sets at weeks 4, 8 and 14, respectively, compared to baseline ($p < 0.05$). Of these, 11, 16 and 1 corresponding genes were up or down-regulated by greater than 2-fold at weeks 4, 8 and 14, respectively. Consistent changes in six of these genes were present at both weeks 4 and 8 and these genes are involved in biological process-metabolism (n=3), cellular component-golgi stack (n=2) and molecular function-lipid binding (n=1) and -catalytic activity (n=2), using Gene Ontology.

This data indicates that GH induces the expression of genes in peripheral leukocytes during GH treatment. Since peripheral blood is easily accessible, identification of a gene expression fingerprint in leukocytes could lead to the development of a GH doping test.

(Funding support from the World Anti-Doping Agency)

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GENERATION AND VALIDATION OF AN FSH-β-PROMOTER CONSTRUCT TO TARGET CRE RECOMBINASE EXPRESSION TO ANTERIOR PITUITARY GONADOTROPES

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Activins and inhibins are members of the TGF-β superfamily which positively and negatively regulate pituitary follicle stimulating hormone (FSH) synthesis respectively. FSH is composed of an α and a β subunit, the latter of which is produced exclusively in pituitary gonadotropes. This expression pattern makes the FSH-β promoter ideal for targeting gene-specific expression in the gonadotropes. The currently available pituitary-specific Cre transgenic mouse is the alpha-GSU-Cre which expresses Cre recombinase in all the five cell types of the adult anterior pituitary. No gonadotrope-specific Cre transgenic mouse has been described. To enable the future creation of conditional gonadotrope-specific gene knockout mice, we are engineering a transgenic mouse line which expresses Cre recombinase mediated by the FSH-β promoter. To generate the FSH-β-Cre construct, a 5.5kb fragment of the ovine FSH-β promoter was fused to the Cre recombinase gene in the pGL3-basic vector. To confirm that the oFSH-β promoter is functional in gonadotrope cells, the same promoter driving luciferase gene expression (oFSHβ-lux) was transfected into the LβT2 mouse gonadotrope cell line. Activin (1 nM) increased the activity of this promoter 5-fold compared to untreated cells. Inhibin (0.5 nM) suppressed this activity by 52 ± 11%. The oFSH-β-Cre construct was tested *in vitro*, by co-transfecting LβT2 cells with a Cre reporter construct (pSV-paX1), containing a floxed stop codon that prevents transcription of β-galactosidase. Cre activity will excise the stop codon, allowing β-galactosidase to be expressed. Treatment of LβT2 cells with 1 nM activin for 24 h, increased the proportion of β-galactosidase-positive cells compared to untreated controls, confirming that activation of the FSH-β promoter is driving Cre expression in these cells. This construct will be used to create transgenic mice for future studies of inhibin and activin actions in the pituitary.

Supported by NH&MRC (Regkeys #241000, 338616 & 198705)

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PREGNANCY-INDUCED LEPTIN RESISTANCE INVOLVES A LOSS OF APPETITE SUPPRESSION BY ALPHA-MELANOCYTE STIMULATING HORMONE

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Despite elevated plasma leptin concentrations, food intake and fat deposition is increased during pregnancy. In fact, during pregnancy a state of leptin resistance develops. We have demonstrated that intracerebroventricular (i.c.v.) leptin administration is unable to suppress food intake in pregnant rats, as it does in non-pregnant animals. One of the major neuronal populations involved in mediating leptin action in the hypothalamus are the proopiomelanocortin neurons in the arcuate nucleus, which produce the anorectic peptide αMSH. Leptin-induced phosphorylation of STAT3 (pSTAT3) in αMSH neurons is normal during pregnancy, suggesting that factors downstream of αMSH may be altered to account for the leptin resistance seen in pregnant animals. To test this hypothesis, we examined the effect of i.c.v. αMSH on food intake in diestrous (n=16) and day 14 pregnant (n=16) Sprague Dawley rats. Infusion cannulae were surgically implanted into the rats, followed by daily food intake measurements during a recovery period of about one week. On diestrus and day 14 of pregnancy, after a 24-hour fast, αMSH (10 μg) or vehicle (aCSF) was injected into the left lateral ventricle. One hour later, at the time of lights off, food was returned and food intake was measured 3 and 24 hours later. During the 24 hours preceding treatment, food intake was significantly greater in pregnant rats compared to cycling rats. In diestrous rats, αMSH treatment (n=8) resulted in significantly reduced food intake compared to vehicle-treated diestrous rats (n=8) both 3 and 24 hours later. In the pregnant rats however, there was no difference in food intake between the αMSH-treated or vehicle-treated rats. These results indicate that not only is pregnancy characterised by leptin resistance, but it is also an αMSH-resistant state. This loss of response to αMSH would therefore contribute to the hyperphagia of pregnancy, an important biological adaptation to help the mother prepare for the metabolic demands of lactation.

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CUSHING'S SYNDROME IN A DIABETIC CLINIC POPULATION

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Introduction: The diagnosis of Cushing's syndrome (CS) is often not straight forward. However, it is important to recognise and diagnose this condition as there is significant morbidity associated with the disease. Recent publications have suggested that a significant proportion of patients with type 2 diabetes mellitus (T2DM) may have undiagnosed occult CS and hence it may be worth screening for CS in patients with DM.[1]

Methods: Overweight patients (BMI>25) (n= 179) with T2DM who had no history of alcohol abuse or psychiatric illness, were recruited from our diabetes clinics. 171 were evaluated with the low-dose (1mg) dexamethasone suppression test (DST). The DST was considered positive if the morning plasma cortisol was greater than 50nmol/L. These patients were further evaluated using 24-hour urinary free cortisol (UFC) collection.

Results: A positive DST was recorded in 31/171 patients. Of these, 3 had elevated UFC. Clinical follow-up identified that 2 of these patients had excessive alcohol consumption as a likely cause of their abnormal investigations. The third patient had 4 UFC collections, 3 of which were elevated. He does not have any stigmata of CS and subsequent radiological investigations have not revealed any pituitary or adrenal pathology.

There were 27 false-positive DST. We postulate that there are a number of mechanisms which may contribute to this including: use of medications metabolised by the cytochrome P450 3A4 (eg Statins, carbamazepine), non-alcoholic hepatic steatosis, obesity, or poor glycaemic control. Interestingly, we did not find any correlation between either BMI and DST cortisol level ($r^2 = 0.017$) or glycosylated haemoglobin and DST cortisol level ($r^2 < 0.001$) in our cohort.

Conclusion: We were unable to identify any individuals from our cohort who had occult CS in the context of documented T2DM. Our experience suggests that widespread screening of asymptomatic diabetic populations is not justified.

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PROGESTERONE WITHDRAWAL TRIGGERS INCREASED PROLACTIN SECRETION AND INDUCTION OF SOCS mRNA IN THE ARCUATE NUCLEUS DURING LATE PREGNANCY

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Under normal conditions prolactin stimulates hypothalamic dopaminergic neurons. In turn, dopamine inhibits prolactin secretion; thereby prolactin regulates its own secretion via negative feedback. During late pregnancy hypothalamic dopaminergic neurons become unresponsive to prolactin, resulting in the antepartum prolactin surge. Suppressors of cytokine signalling (SOCS) proteins have been implicated in mediating this change of dopaminergic neuron response. Around day 20 of pregnancy, progesterone concentrations decrease while estrogen remains high. We hypothesized that the initiation of the prolactin surge and consequential increased SOCS expression is triggered by progesterone withdrawal. Thus, by delaying the fall in progesterone during late pregnancy we should abolish changes in prolactin regulation and SOCS mRNA expression. Sprague Dawley rats were ovariectomized on day 18 of pregnancy and received progesterone and estrogen implants. Progesterone implants were removed on day 21, to mimic the fall in progesterone, or day 22 of pregnancy, to delay the fall in progesterone by 24 hours. Control animals remained intact. We observed a significant increase in prolactin during day 22 compared to day 18 of pregnancy in intact animals (123.5 ± 18.0 ng/ml vs. 6.7 ± 1.3 ng/ml; $P < 0.05$). Following progesterone withdrawal on day 21 we observed a similar antepartum prolactin surge, however it was not as marked as in intact animals (53.6 ± 7.6 ng/ml; $P < 0.05$). No surge was observed following delayed progesterone withdrawal. In addition, we observed a 3-fold increase ($P < 0.05$) in SOCS-1 and CIS mRNA levels in the arcuate nucleus during the prolactin surge in control animals and following progesterone withdrawal on day 21. No significant change in SOCS mRNA was seen following delayed progesterone withdrawal. These results support our hypothesis that the timing of the antepartum prolactin surge and the associated change in arcuate SOCS-1 and CIS mRNA levels depend on the withdrawal of progesterone.

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IMPACT OF NEONATAL INFECTION ON CRH AND GLUCOCORTICOID RECEPTOR mRNA ABUNDANCE IN THE MOUSE BRAIN.

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Background: Prenatal and neonatal infection has been shown to alter glucocorticoid receptor function and the adult stress response in a number of animal models. However no studies have examined whether neonatal infection also alters the stress response in the mouse. The current study aims to examine alterations to glucocorticoid receptors (GR), mineralocortoid receptors (MR) and corticotropin releasing hormone (CRH) mRNA after neonatal exposure to Chlamydia in the mouse.

Method: At birth neonates were exposed to Chlamydia Pneumoniae (intranasally, 400 ifu). Control animals received no treatment. At six and nine weeks old animals were euthanised and brains removed and snap frozen. RNA was extracted and GR, MR, and CRH abundance was measured with β actin as the reference gene using real-time polymerase chain reaction.

Results: Neonatal infection resulted in a significant ($p = 0.01$) decrease in MR and CRH abundance when compared to controls for both male and female mice. Male mice had a significant ($p = 0.01$) decrease in MR and CRH compared to female mice in response to neonatal infection. There was no change in GR abundance in response to neonatal infection in either male or female mice.

Discussion: The current study suggests that changes to the stress response in the mouse may be mediated by alterations to MR and CRH rather than GR at the level of mRNA. The current study suggests that the mouse is a suitable model to examine the role of neonatal infection in programming alterations to the adult stress response.

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ACTIVATION OF CORTICOTROPHIN-RELEASING HORMONE (CRH), ARGININE VASOPRESSIN (AVP) AND ENKEPHALIN (ENK) NEURONES LOCATED IN THE PARAVENTRICULAR NUCLEUS BY ISOLATION AND RESTRAINT STRESS IN SHEEP

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The hypothalamo-pituitary-adrenal (HPA) axis is activated during stress and there are sex differences in the activation of the HPA axis which are reflected in plasma concentrations of cortisol. The basis for these differences is not known. Corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) producing neurones of the paraventricular nucleus (PVN) are activated during stress and control the HPA axis. Enkephalin producing neurones of this region are also activated during stress. We tested the hypothesis that there is a sex difference in the activation of CRH, AVP and enkephalin neurones during isolation/restraint stress in sheep. Blood samples (3h) were taken to monitor plasma cortisol concentrations and stress was imposed for 1.5h after 1.5h of control blood sampling. The brains of gonadectomised male and female sheep (n=3/sex/group) were collected after isolation/restraint stress (stress group) and from contemporaneous controls. Double-labelling immunohistochemistry for Fos and either CRH, AVP or enkephalin was undertaken to quantify the number of each type of neurones that was activated during stress. No sex differences were observed in cortisol concentrations in control or stressed animals. Isolation and restraint caused an increase in the number of CRH and AVP cells with Fos-immunoreactive (-ir) nuclei without sex differences. The number of enkephalin cells with Fos-ir positive nuclei was increased in male but not female sheep. We conclude that isolation/restraint stress activated similar numbers of CRH and AVP neurones in both sexes but activates enkephalin neurones in males only.

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THE EFFECTS OF A HIGH PHYTOESTROGEN DIET ON THE TESTES OF THE RAT

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Environmental oestrogens have been suggested to have deleterious effects on male reproduction. This study investigated the effects on the testes of exposure to a high phytoestrogen (PO) diet at various stages in the development of the rat. Male rats were exposed to a high PO diet (465microgram/g) from either: conception to birth, birth to weaning, weaning to adulthood or from conception to adulthood. Control animals received a low PO diet (112 microgram/g) from conception to adulthood. Groups of male rats (n=10) were killed at 18 days, 6 and 16 weeks postpartum and the testes removed for histology and subsequent stereological analysis.

At 18 days spermatocytes were present in all testes, but spermatids were only identified in animals exposed to a low PO diet during conception. At 6 weeks significantly less residual bodies were present in tubules of animals that had received a high PO from conception ($P < 0.05$). Epididymal sperm numbers were also lower in these animals.

No differences in the number of Leydig cells were observed between any of the groups at 16 weeks. A significant reduction ($P<0.01$) in the number of Sertoli cells was observed in animals exposed to a high PO diet continually from conception. These animals also had reduced numbers of spermatogonia ($P<0.002$) and spermatocytes ($P<0.04$) compared to the control animals, however, no significant difference in the number of spermatids was observed. No changes in Sertoli or germ cell number were seen in any of the other treatment groups.

These data suggest that a high PO diet during pregnancy and/or lactation may affect the onset of puberty. Continuous exposure to a high PO diet from conception may affect the number of Sertoli cells, spermatogonia and spermatocytes in the adult animal. However, mechanisms appear to exist to prevent significant reduction in postmeiotic germ cell numbers.

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DO EXOSOMES FROM PLACENTAL EXPLANTS CARRY SYNCYTIN?

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Background: In general, retroviral infection has been related to immunosuppression and it has been shown that the cause of this immunosuppression is the presence of a highly conserved immunosuppressive peptide (ISU) in the transmembrane subunit (TM) of the retroviral envelope protein. Human placenta expresses high levels of endogenous retroviral envelope protein and the presence of these proteins have been hypothesised to promote cell-cell fusion during placental formation and immunosuppression during pregnancy. In particular, Syncytin, a human endogenous retrovirus envelope protein belonging to the HERV-W family is expressed at high levels in the placenta and has been

demonstrated to have fusogenic properties as well as a putative ISU within the TM of the protein. We raised antibodies to target the putative ISU of Syncytin. We hypotesised that the human placenta may produce exosomes that express the retroviral protein Syncytin and that expression of this protein may be regulated by cAMP.

Methods: Placental explants were collected and cultured for 24 hours and then treated with Forskolin (50 mM), CRH (100nM), and a combination of Forskolin (50 mM) and CRH (100nM) for 24, 48 and 72 hours at 37^{o}C (5% CO_2). At each end point, the supernatant was removed and exosomes were enriched by ultracentrifugation, proteins extracted and analysed using Western blotting to detect the presence of Syncytin's TM subunit.

Results: Western Blotting analysis of enriched placental exosomes showed cross-reactivity with a 24kDa protein, the expected size of Syncytin's TM subunit, which increased in a time dependent manner and appeared to be independent of Forskolin or CRH treatments. Also, preliminary observations show cross-reactivity of our antibody with a 24kDa protein present in human plasma from pregnant women.

Conclusions: Using antibodies we raised to target the putative ISU of Syncytin, we found cross-reactivity with a 24kDa protein corresponding to the expected molecular weight of the TM subunit of Syncytin in exosomes produced by human placental explants. No increase in Syncytin expression in an exosomal fraction was observed in response to agents known to increase cAMP. These results suggest a novel mechanism by which the placenta may influence the immunology of pregnancy.

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TRANSGENERATIONAL EFFECTS OF DEVELOPMENTAL PROGRAMMING ON THE FETAL AND PLACENTAL CHARACTERISTICS OF THE RAT.

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Maternal under-nutrition during early gestation can alter both fetal growth rate and endocrinology. Fetal growth restriction during pregnancy is associated with an increased risk of disease in adult life, particularly type II diabetes. The current study focuses upon the effects of developmental programming on the reproductive potential of the subsequent generation. Female Wistar rats were assigned to receive either a standard diet *ad libitum* (AD group) or 30% of the *ad libitum* standard diet (UN group) throughout gestation. Litter size was standardised and female offspring from these pregnancies (F1 generation) were then fed the standard diet *ad libitum* until day 140. At this time these females were mated with AD males and subjected to one of the two dietary regimes described during gestation. A cohort of pregnancies was terminated at day E20 with fetal and placental parameters recorded. When the remaining animals gave birth their litter size was standardised and the F2 pups (males and females) were nursed by their dams until weaning. This produced 3 groups of offspring (F2 generation) (AD-AD, AD-UN, UN-AD). Despite being fed *ad libitum* since weaning F1 UN-AD dams demonstrated significant ($P<0.05$) hyperphagia during both pregnancy and lactation relative to control AD-AD dams. Moreover, fetal reabsorptions were significantly increased (more than 3 fold higher) in UN-AD compared to AD-AD dams. Pup weights were not different between these treatments, however, placentae of UN-AD pups were significantly smaller ($P<0.05$) and consequently the fetus to placenta ratio of these pups was equivalent to AD-UN rather than AD-AD pups. In addition, the litters of UN-AD dams were significantly skewed ($P<0.05$) towards males (59%) compared to AD-AD litters (50%). These results clearly identify transgenerational effects of developmental programming on maternal appetite and reproductive characteristics. *Supported by Maurice and Phyllis Paykel Trust.*

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DIFFERENTIAL EXPRESSION OF THE BETAGLYCAN GENE IN THE MURINE OVARY AND TESTIS DURING GONADOGENESIS.

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Betaglycan (BG) binds inhibin and transforming growth factor-beta (TGF-β) with high affinity and is a key modulator of the actions of many TGF-β superfamily members. Abundant expression of BG is observed in both sexes in the adult gonads, suggesting important roles in regulation of reproduction in both the male and female. The significance of this cell surface receptor during gonadogenesis is yet to be elucidated. In this study, we characterised the gonadal expression pattern of BG during murine embryonic development ($n\geq2$ for each study). The expression of BG mRNA was detected using wholemount in situ hybridisation in the bipotential gonad at day 11.5 days post-coitum (dpc) and by semi-quantitative reverse transcription polymerase chain reaction in male gonads at 12.5-18.5 dpc. Low levels of BG mRNA were detected in the developing female gonad at 12.5-14.5 dpc, with reduced expression at later ages prior to birth. Both the male and female gonads expressed BG on the day of birth, neonatally and in adulthood.

Immunohistochemistry data indicated that BG protein was low in the embryonic ovary compared to the developing testis, for which protein expression was detected in interstitial somatic cells from 12.5-16.5 dpc. Expression was also localised to the cells surrounding the developing seminiferous cords. Low BG protein expression was detected in the male germ cells and pre-Sertoli cells. These data indicate that BG is differentially expressed between the sexes during gonadogenesis, and suggest a role for BG in male gonadal differentiation.

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DIFFERENCES IN TUMOUR NECROSIS FACTOR ALPHA PRODUCTION BY TERM AND PRETERM PLACENTAE

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Background: Placental cytokines may play a role in the initiation of spontaneous labour in term and preterm pregnancies. The production of tumour necrosis factor (TNF) alpha and other Th1 cytokines by the placenta are associated with chorioamnionitis and preterm delivery [1]. It has been observed that TNF alpha levels are higher in placentas from normal deliveries compared to preterm and premature rupture of membrane deliveries [2]. We were interested in characterising the response to an inflammatory challenge in placentae collected from term and preterm deliveries and whether there were any differences in the inhibitory effect of glucocorticoids on this pathway with gestational age.

Methods: Placentae were collected from normal term deliveries and preterm deliveries of pregnancies complicated by pre-eclampsia and IUGR. Placental explants were cultured for 24hrs and then exposed to lipopolysaccharide (LPS) (10μg/ml), in the presence and absence of 100nM dexamethasone, 1μM cortisol or 10μM cortisone. After 24hrs the supernatants were assayed for TNF alpha by sandwich ELISA.

Results: Placentae from term deliveries had a significantly higher TNF alpha response to LPS than preterm placentae ($p<0.05$). Glucocorticoid inhibition of placental TNF alpha production was significantly greater in term placentae than preterm placentae ($p<0.05$). Cortisone did not have any effect on term or preterm placental TNF alpha response.

Conclusion: These data suggest that placental TNF alpha production in response to inflammation increases with increasing gestational age. The inhibition of this response by glucocorticoids is significantly greater in term than preterm placentae suggesting an inflammatory challenge in preterm placentae may be significantly more detrimental.

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PLACENTAL GENE EXPRESSION IS ALTERED BY FETAL SEX, MATERNAL ASTHMA AND INHALED GLUCOCORTICOID INTAKE

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Background: Previous research examining the effect of maternal asthma during pregnancy on placental function and fetal outcome indicated there were sex specific differences in how the fetus responds to maternal asthma. These data suggested male and female fetuses initiate different mechanisms to the same stress and raised the question of whether there were global differences in placental gene expression in relation to fetal sex, maternal asthma and inhaled glucocorticoid treatment.

Methods: Using microarray we determined the gene expression profiles of placentae collected from male or female fetuses of normal, human pregnancies and pregnancies complicated by asthma in the presence and absence of inhaled glucocorticoid intake. Data was analysed using a Binary Tree Structured Vector Quantization algorithm which generates a gene expression map. Sites on the map where there were obvious differences in gene expression were selected for analysis.

Results: Placentae from female fetuses of asthmatic mothers that did not use inhaled steroids during pregnancy had 37 gene alterations relative to the control population. Placentae from male fetuses of asthmatic mothers that did not use inhaled steroids during pregnancy had 6 gene changes relative to the control population. Placentae from female fetuses of asthmatic mothers who did use inhaled steroids during pregnancy had 22 gene alterations relative to the control population and placentae of male fetuses had no gene changes. There were 10 placental genes altered in the presence of maternal asthma that were common to both male and female fetuses.

Conclusion: This data indicates that there are significant differences in how a placenta from a male fetus and female fetus respond to a maternal disease and raises the question of whether we are significantly compromising our interpretation of human placental data when we do not take the sex of the fetus into account.

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LOBE-SPECIFIC REDUCTION IN MATURE PROSTATE STRUCTURAL AND FUNCTIONAL DIFFERENTIATIATION IN PROSTATE EPITHELIAL SPECIFIC ANDROGEN RECEPTOR KNOCKOUT (PEARKO) MICE

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A functional androgen receptor (AR) is crucial for mesenchymal cell-dependent paracrine pathways that induce prostate development and cellular differentiation, but the distinct roles of AR in stromal and epithelial cells in the mature prostate remains to be defined. To study the physiological function of prostate epithelial AR in the mature prostate, we established a novel mouse model with targeted disruption of prostate epithelial AR. Floxed-AR allele carrying mice (exon3 flanked by loxP sites) were crossed with transgenic probasin promoter-driven Cre (Pbsn-cre) mice to generate epithelial-specific AR knockout (PEARKO) mice. Eight week old PEARKO males had significantly ($p<0.05$) decreased weight of all prostate lobes (36-80% of control), while serum testosterone levels and testis weight were unaltered. Functional cytodifferentiation of PEARKO prostate epithelial cells was reduced ($p<0.05$) in all lobes with most prominent effects in dorsolateral and anterior lobe epithelium. To evaluate the prostate structural differentiation, real-time RT-PCR was used to analyze mRNA abundance of cytokeratin 8 (CK8) for mature luminal epithelial cells and smooth-muscle α-actin (SMA) for smooth muscle. Real-time RT-PCR results were normalized against cyclophilin mRNA expression. Relative abundance of CK8 was unaltered while SMA was significantly ($p<0.05$) decreased in anterior and dorsolateral prostate, 46 and 53% of control, respectively. For functional cytodifferentiation, mRNA abundance was analyzed for prostate lobe-specific, androgen-dependent secretory protein genes: renin-1, probasin, and MP25 for anterior, dorsolateral and ventral prostate, respectively. The most significant changes were observed in anterior and dorsolateral prostates while ventral prostate was least affected. Relative expression of androgen responsive, lobe specific mRNA for AP, DLP and VP was significantly ($p<0.05$) decreased 72, 88 and 39% respectively. These results indicate that epithelial AR is required to maintain full prostate structural and functional differentiation, highlighting that prostate lobes are distinct in their morphology and hormonal responsiveness. (Funded by Cure Cancer Australia)

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UTERINE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR-A mRNA IN HORMONE TREATED OVARIECTOMISED MICE

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Endometrial angiogenesis induced by oestrogen and progesterone is mediated by vascular endothelial growth factor (vegf-A) but the molecular mechanisms involved remain unclear. In this study we quantified relative changes in mRNA expression levels of total vegf-A, the individual vegf-A isoforms (120, 144, 164, 188 and 205) and the vegf-A receptors (flk-1 and associated receptors nrp-1 and nrp-2) in whole mouse uteri following oestrogen and progesterone treatment. *Animal Models: Progesterone Regime*: Mice (CBA x C57, n=9) were treated with a single injection of 100 ng of estradiol on day eight following ovariectomy, followed by a day with no treatment and three consecutive daily injections of 1 mg progesterone. Two groups were treated with either the vehicle (n=5) or progesterone only (n=10). All mice were dissected on day 13 after ovariectomy. *Short-term Oestrogen Regime:* Mice (n=9) were given a single injection of 100 ng of estradiol and dissected 24 hours later. mRNA expression was quantified by real time RT-PCR. All results were normalized against 18S rRNA. Relative levels of total vegf-A, flk-1, nrp-1 and nrp-2 mRNA were significantly lower ($p=0.01$) in those animals dissected 24 hours following oestrogen treatment compared with the mice treated with vehicle or progesterone only. Although there was no significant difference in the mRNA expression levels of the vegf-A isoforms 120, 164 and 188 between treatment groups, the highest levels of mRNA expression were detected for vegf 164 and the lowest levels for vegf 188. vegf-A 144 and 205 were not detected. We conclude that treatment of ovariectomized mice with oestrogen produces a concurrent reduction in total vegf-A, flk-1, nrp-1 and nrp-2 mRNA expression in whole uterine tissue.

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DEVELOPMENTAL EXPRESSION OF PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS α AND γ IN THE BOVINE PLACENTA

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Peroxisome proliferator-activated receptors (PPARs) constitute a subfamily of nuclear hormone receptors that are involved in lipid metabolism, differentiation, proliferation and inflammation. PPAR α is expressed in highly oxidative tissues, and plays a key role in regulation of cellular uptake, activation and β-oxidation of fatty acids. PPAR γ is present in adipose tissue, and promotes adipocyte differentiation and lipid storage. Fatty acids (FAs), FA-derived compounds and eicosanoids such as prostaglandins are natural ligands for both PPARs. Gene expression of PPARs α and γ have been reported in human and rodent placenta and potentially play a role in placental development, but their role in bovine pregnancy is unclear.

This study aims to investigate the placental gene expression patterns of PPARs α and γ during early bovine pregnancy. Partial bovine PPARs α and γ genes were successfully cloned using RT-PCR and mRNA expression levels measured in developing bovine placenta (fetal cotyledons and maternal cotyledons) at Days 50, 100 and 150 gestation using RT-PCR and northern blot analyses. In maternal cotyledons, PPAR α expression peaked at Day 100 of gestation whereas very low levels were observed at Days 50 and 150. Very low levels of PPAR α expression was also detected in Day 150 fetal cotyledons. PPAR γ expression was observed only in maternal cotyledons at Day 50, and was present in both fetal and maternal cotyledons at Days 100 and 150 of gestation.

The presence of PPAR α and γ in the bovine placenta during early gestation suggest that both PPARs maybe required for metabolism of fatty acids in the bovine placenta. Our work provides a framework for the further investigation of PPARs and their specific role in regulation of placental development and function to ensure successful bovine fetal development.

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STEROIDOGENIC POTENTIAL OF THE BOVINE FETAL ADRENAL AT EARLY TO MID-GESTATION

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Steroid hormone synthesis from cholesterol involves the activity of several enzymes. During fetal development, the placenta and fetal adrenal complement each other in steroidogenic activity during pregnancy. This presentation focuses on determining the ontogeny of steroidogenic potential in the fetal bovine adrenal during early to mid-gestation. Fetal adrenal tissues were collected between Days 138 and 165 of gestation (full term ~270 days) from bovine fetuses generated by artificial insemination. The expression of the steroidogenic enzyme genes involved in glucocorticoid, mineralocorticoid and sex steroid synthesis were investigated in the fetal adrenal gland using Northern blotting with total RNA. The genes examined were: cholesterol desmolase (*Cyp11A*), 17α-hydroxylase (*Cyp17*), 3β-hydroxysteroid dehydrogenase (*3β-HSD*), 11β-hydroxylase (*Cyp11B*), steroid 21-hydroxylase (*Cyp21*) and cytochrome p450 aromatase (*Cyp19*). The cellular localization of these mRNAs was determined by *in situ* hybridization with digoxigenin-labelled probes. *Cyp11A*, *3β-HSD*, *Cyp21* and *Cyp11B* were all expressed in fetal adrenals from Days 138 to 165. There was no detectable *Cyp17* or *Cyp19* mRNA in the fetal adrenals at these stages. *In situ* hybridization showed that *Cyp11A*, *Cyp11B*, *Cyp21* and *3β-HSD* mRNA were all localized to the developing fetal adrenal cortex. Thus, *de novo* synthesis of progesterone, corticosterone and aldosterone from cholesterol can occur in the fetal adrenal from around mid-gestation. However, the lack of *Cyp17* and *Cyp19* expression suggests that the bovine fetal adrenal cannot synthesize cortisol and estrogens at this stage. The placenta likely complements the adrenal gland with the synthesis of the sex steroids, progesterone and estrogen, during bovine fetal development.

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THE IMPACT OF ANTENATAL CORTICOSTEROIDS ON UMBILICAL VENOUS AND ARTERIAL CORTISOL IN NEONATES 23-41 WEEKS GESTATION

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Aim: Corticosteroids are employed clinically to improve newborn outcome in cases determined to be at risk of preterm birth. We have previously demonstrated that there is a sex specific response to a change in cortisol concentration in term neonates. We investigated the effect of antenatal glucocorticoids on umbilical cortisol in preterm neonates in relation to birth weight, gestational age and sex.

Methods: Preterm infants (n=61) exposed (n=28) or not exposed to antenatal glucocorticoids within 10 days of delivery, were studied. Umbilical venous (UV) and arterial (UA) cortisol was determined by radioimmunoassay.

Results: Cortisol levels in UA and UV showed a positive relationship (r^2=0.677, p<0.0001). The betamethasone exposed group (A) had a lower gestational age (p=0.0001), birth weight (p=0.001), and UV cortisol (125.58 ± 33 nmol/l vs 251.47 ± 42.9 nmol/l, p=0.09) when compared to the non-betamethasone exposed group (B). In A there was no relationship between UV cortisol and birth weight in male infants but female infants demonstrated a negative correlation (r^2=0.284, p=0.06). In B there was a positive correlation between UV cortisol and gestational age (r^2=0.3316, p=0.0005) and UV cortisol and birth weight (r^2=0.3195, p=0.0004). There was no relationship between fetal sex, birth weight and UV cortisol in B.

Conclusion: Preterm neonates not exposed to betamethasone before delivery had increased umbilical venous cortisol concentrations with advancing gestation and birth weight. The administration of maternal betamethasone resulted in a sex-specific response in relation to birth weight and UV cortisol. Further investigation of sex-specific effects of maternal betamethasone on the fetal HPA and its down-stream actions is warranted.

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DILATED CARDIOMYOPATHY IN A PATIENT WITH CUSHING'S SYNDROME

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A 28-year-old lady was admitted with a 3-week history of dyspnoea. Her medical history was unremarkable except for smoking. Examination revealed signs of biventicular failure. Features of Cushing's syndrome, with mild proximal limb weakness, were also present. She does not drink alcohol and had no family history of cardiomyopathy.

Her initial electrocardiogram showed sinus tachycardia with left atrial enlargement and chest X-ray revealed cardiomegaly and pulmonary oedema. Transthoracic echocardiography showed dilated left ventricle, ejection fraction (EF) of 34% and mild mitral regurgitation, consistent with dilated cardiomyopathy. No auto-immune or infective cause for the cardiomyopathy was found.

Twenty-four hour urinary free cortisol was markedly elevated (964 nmol/24 hours; reference range: 50-350 nmol/24 hours), with suppressed corticotropin (ACTH). Computed tomography of the abdomen showed a 3.8 x 2.3 cm right homogenous adrenal mass with low attenuation. Therefore findings were consistent with ACTH-independent Cushing's syndrome, most likely due to adrenal adenoma.

Her cardiac failure was treated with ramipril, carvedilol, spironolactone and frusemide. However due to her poor cardiac function, adrenalectomy had to be delayed. In the interim, she was treated with ketoconazole to inhibit steroidogenesis. Three months after ketoconazole treatment, urinary free cortisol level was lower (229 nmol/24 hours).

Six months after treatment, her symptoms improved; repeat echocardiography showed reduction in left ventricular size and EF improved to 50%. She then underwent right adrenalectomy. Pathology confirmed a benign adrenal adenoma. She developed postoperative tertiary adrenal insufficiency and required hydrocortisone therapy. Six months after surgery, features of Cushing's syndrome were resolving and repeat echocardiography showed normal left ventricular size and EF (67%).

Reversible dilated cardiomyopathy is a rare complication of Cushing's syndrome, reported only in two previous cases. Although skeletal myopathy is a well recognised complication of Cushing's syndrome, our knowledge of cardiomyopathy in this setting is more limited. A literature review of cardiac function in Cushing's syndrome will be discussed.

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ACROMEGALY AND THYROID SIZE

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AIM: To assess the prevalence of thyroid goitre in individuals with acromegaly and to determine the effect of successful treatment on thyroid size.

Method: A retrospective analysis was conducted of individuals receiving treatment in Westmead Hospital from 1994 to 2005. Each individual had to have at least two thyroid ultrasounds, along with serial measures of IGF-1 and TSH. RESULTS: Results are presented as mean and SD. 9 individuals (7 male, 2 female) were included in the sample. They had a mean duration of disease of 14.67 + 6.25 yrs. While all received Octreotide LAR treatment, they differed in prior treatment modalities. Initial and current results include: TSH: 1.66 +/- 1.96mU/L vs. 1.02 + 0.74mU/L; IGF-1: 70.78 +/- 34.01nmol/L vs. 28.71 +/- 9.64nmol/L. The only significant difference was in IGF-1 levels (t-score=3.57, p<0.01). The initial thyroid ultrasounds showed Thyroid Volume (TV): 23.59 +/- 13.73mls. The current thyroid ultrasounds showed

TV: 25.30 +/- 15.17mls. There were no statistically significant correlation between initial and current TVs with respect to TSH and IGF-1. Disease control was defined as >75% of an individual's recorded IGF-1 being < 45nmol/L. Those with good control (n=6) were compared to those with poor control (n=3). Initial and current TV for the good control group was: 18.97+/- 14.82mls vs. 22.25 +/- 13.60mls. Initial and current TV for the poor control group was: 32.78 +/- 3.85mls vs. 31.42 +/- 19.37mls. There was a significant difference in the initial TVs between good and poor control (t-score=2.14, $p<0.05$).

DISCUSSION: Acromegaly was associated with increased thyroid size. There was no regression of thyroid size despite adequate control of IGF-1. Between those with good and poor control, there was no difference in current TVs. However, those with poor control longitudinally had improved IGF-1 levels which may explain the lack of difference. There is scope for larger cohort studies to follow the progression of the thyroid in adequately treated acromegaly.

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THE USE OF PRE-OPERATIVE ULTRASOUND MAPPING OF CERVICAL LYMPH NODES TO GUIDE SURGERY FOR PERSISTENT AND RECURRENT PAPILLARY THYROID CARCINOMA

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Cervical lymph node (LN) metastases from papillary thyroid carcinoma (PTC) are associated with an increased loco-regional recurrence rate. Surgery remains the therapeutic modality of choice for resectable neck disease in the absence of widespread metastases and in the context of negative whole body scan (WBS). The use of routine prophylactic neck dissection is unpopular with data suggesting that it has no effect on disease-specific mortality. Techniques for LN dissection include "berry-picking" (not advocated for initial surgical treatment of PTC lymphatic metastases), selective LN dissection (a targeted lymphadenectomy more relevant to the nature of the spread of PTC) and modified radical LN dissection (levels I-V are removed). The ideal surgical method to reduce morbidity from recurrent laryngeal nerve neuropraxia and improve surgical outcomes remains uncertain. In recent years several strategies have been used to guide and improve the accuracy and outcome of surgical resection of persistent or recurrent disease including pre-operative neck ultrasound mapping, sentinel node detection using "blue dye", therapeutic ^{131}I followed by radio-detector probe-guided surgery and intra-operative ultrasound.

We describe our institution's experience with pre-operative ultrasound mapping of loco-regional neck disease in patients with PTC in the context of elevated thyroglobulin and negative WBS. High resolution ultrasound using a 10-14MHz probe is performed and the location of abnormal nodes is marked on the skin using a surgical pen with indelible ink. When the patient is draped at surgery, a sterile clear plastic film is fixed over the operative field and marked with "X"s corresponding with the skin marks. This film can be replaced over the field at any time to guide surgical dissection. Intra-operative ultrasound is used to guide the surgeon to the node under the "X" to ensure all abnormal tissue is removed. This technique may prove to have significant impact on surgical ou tcomes.

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THE DIAGNOSTIC VALUE OF NECK ULTRASOUND AND THYROGLOBULIN MEASUREMENT IN THE FINE NEEDLE ASPIRATE FROM CERVICAL LYMPH NODES IN THE FOLLOW-UP OF PATIENTS WITH PAPILLARY THYROID CARCINOMA

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Papillary thyroid carcinoma (PTC) is the most frequent histological type of differentiated thyroid cancer, with neck lymph node metastases found in up to 70% of cases. TSH-stimulated thyroglobulin (Tg) and whole body scan (WBS) are currently the main surveillance paradigms. However Tg positive, WBS negative disease necessitates further imaging which includes computerised tomography (limited in identifying subcentimetre lymph nodes), ^{18}FDG-PET scan (expensive) and neck ultrasound. Features of metastases on ultrasound include reduced internal echoes, non-homogeneity, a rounded or bulging shape, an absent hilar echogenic line, height : width ratio >0.5 in the transverse view, microcalcification or a cystic component and increased internal vascular signature. The diagnostic usefulness of fine needle aspiration cytology (FNAC) of metastatic neck lymph nodes is limited by the presence of cystic change, which can occur in up to 70% of metastatic PTC (1). Thyroglobulin measurement in the elute from fine needle aspiration (Tg-FNA) is an emerging technique used to diagnose neck metastases and recurrence. Tg-FNA is not affected by the presence of serum Tg antibodies (2). This modality combined with cytology increases the sensitivity of detecting PTC metastases from 76% to 100% (3,4).

We report our institution's experience of Tg-FNA with a small series of eight patients with PTC who have undergone both FNAC and Tg-FNA of suspicious neck lymph nodes detected at follow-up on ultrasound in the setting of elevated serum Tg and negative WBS. Ultrasound in conjunction with Tg-FNA has proved to be an efficient method of surveillance of these patients. Tg-FNA increases the sensitivity and may be superior to FNAC in detecting lymph node metastases from PTC.

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THE PREVALENCE OF THYROID DISEASE AND RELATED RISK FACTORS IN TASMANIA

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Introduction: Recent studies have disclosed re-emergent iodine deficiency (ID) in Tasmania, as well as previously unrecognized endemic ID in mainland Australian states. However, the prevalence of thyroid disease (TD) and the long-term sequelae of ID in the adult Australian population is poorly characterised.

Aim: To evaluate the prevalence of TD in the adult Tasmanian population using a questionnaire and ultrasonographic methodology.

Methods: A random sample of 10 000 adults registered on the Tasmania electoral role in the year 1999. 5774 (57.7%) respondents aged 18-85 years completed a health questionnaire, of whom 463 were randomly selected to undergo thyroid ultrasound.

Results: An history of TD was reported by 3.9% and 15.7% of male and female respondents respectively. The prevalence of goitre was higher for individuals residing in Tasmania since birth* compared to those not born in Tasmania and living greater than 19 years elsewhere** However, ultrasonography revealed thyroid nodules (particularly multinodularity) to be at least as frequent irrespective of birth place and subsequent State of residence (Table)

Characteristics	Born in Tasmania*		Not born in Tasmania**	
	Male %	Female %	Male %	Female %
Questionnaire (n=5774)	24.6	29.9	9.8	9.1
Age (yrs)	51.9±0.5	51.1±0.4	60.7±0.6	58.3±0.7
Goitre	2.5	7.4	0.9	4.2
Thyroid surgery	1.3	4.5	0.7	3.8
Thyroid cancer	0.4	0.9	0.5	0.6
Hyperthyroid	1.1	4.6	1.4	2.9
Hypothyroid	1.2	5.7	0.9	5.5
On thyroxine	0.6	3.9	0.4	3.3
FHx of thyroid disease	11.1	23.6	8.5	13.0
Ultrasonography(n=205)	18.5	34.6	8.3	12.2
Age (yrs)	52.8±2.6	47.7±1.3	64.8±2.6	60.2±2.1
Thyroid volume (ml)	12.7±0.8	8.6±0.6	10.4±1.3	10.1±0.9
No Nodules	71.1	53.5	58.8	40.0
Nodules	28.9	46.5	41.2	60.0
1 nodule	13.2	11.3	11.8	4.0
Multiple nodules	15.7	35.2	29.4	56.0

Conclusion: TD is common both in Tasmania and the broader Australian population. Subclinical thyroid nodularity is frequent and not restricted regions of historical ID .

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THE INFLUENCE OF GESTATION ON URINARY IODINE EXCRETION IN PREGNANCY

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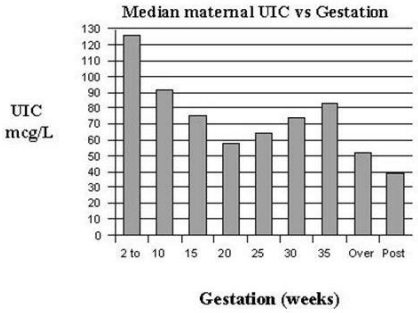
Background: The recommended daily intake of iodine is 200-250 mcg/d during pregnancy, compared to 150mcg/d in non-pregnant adults and 90-120 mcg/d in children. Renal iodine excretion is glomerular filtration rate (GFR) dependant, and GFR increases during pregnancy. Whereas community iodine nutrition is deemed sufficient when median urinary iodine concentration (UIC) in primary school children is ≥100mcg/L, normative ranges for pregnancy have not been established. This study evaluates changes in UIC during pregnancy in a population with documented iodine deficiency (ID).

Methods: 698 urine samples were collected from 461 women attending the antenatal clinic at the Royal Hobart Hospital. The study was conducted between 1998-2001 when median UIC in primary school age children was 84µg/L.

Results: Overall median UIC during pregnancy was 78µg/L at a mean of 22.0 weeks gestation. Stratification by gestation at time of sampling revealed UIC initially increased in early pregnancy, then declined with advancing gestation (Table and Figure).

Gestation (wks)	*n=*	*Median UIC (mcg/L)*	*% women < 50mcg/L*
2 to 9.9	18	126	22.2
10 to 14.9	181	92	24.9
15 to 19.9	168	75	25.0
20 to 24.9	46	58	47.8
25 to 29.9	36	64	27.8
30 to 34.9	59	74	33.9
35 to 39.9	99	83	24.2
over 40 weeks	20	52	46.2
Post partum	71	39	66.2



Conclusion: After an initial GFR related rise in iodine excretion compared to the non-pregnant population, UIC decreases as pregnancy progresses, thus increasing the proportion of women falling into a range indicative of moderately severe ID. These results indicate that assessment of ID in pregnancy requires gestation specific reference ranges. Reporting of non-stratified median UIC in pregnancy is likely to underestimate the severity of ID in pregnant women.

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IMPLEMENTATION AND EVALUATION OF A PROTOCOL FOR REDUCED GLUCOCORTICOID REPLACEMENT IN PITUITARY SURGERY.

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Introduction: For many years the prescription of high doses of glucocorticoids following pituitary surgery has been standard care at RMH and the majority of neurosurgical centres worldwide. This practice may be associated with an unacceptably high incidence of post-operative Cushing's syndrome. A recent review of this practice recommended much lower doses of glucocorticoids, to be given only if there were a high likelihood of post-operative deficiency or biochemical evidence of hypocortisolism.

Aim: To design and implement a protocol of reduced glucocorticoid replacement in pituitary surgery and assess its utility by comparing clinical and biochemical outcomes of pituitary operations performed before (2002-2003; Group A) and after (2004-2005; Group B) its implementation.

Results: 45 and 41 operations were included in Groups A and B, respectively. There were no significant differences in age, gender, underlying pathology or operative approach between the two groups. Frequency of glucocorticoid use was higher in Group A before, during and after surgery. Long-term (beyond eight weeks after surgery) use of glucocorticoids was 79% v 20% in Groups A and B respectively. There was more post-operative diabetes insipidus (29% v 15%) and a similar frequency of adrenal crisis (4% v 5%). The length of stay was higher in Group A (7.2±0.4 v 5.6±0.5 days).

Conclusion: Our protocol of selective glucocorticoid replacement in pituitary surgery is safe and associated with improved clinical outcomes.

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THE EFFECT OF WEIGHT LOSS ON BLOOD PRESSURE, SALT SENSITIVITY AND ADRENOCORTICAL FUNCTION

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Background: The hypothalamic-pituitary-adrenal axis (HPA) and the renin-angiotensin-aldosterone system (RAAS) have been implicated in the pathophysiology of obesity induced hypertension. However, there is little data on the effect of moderate weight loss on the blood pressure response to salt loading and adrenocortical function.

Study design: Twenty five obese subjects (age 39-63 yr, BMI 32.9 ± 4.3 kg/m ^{2}) followed a 12 week weight loss diet before and after which they followed a high (250 mmol/d) or low salt (30 mmol/day) diet for 2 weeks crossed-over and randomised. After each diet, 24-hr ambulatory blood pressure, plasma aldosterone, renin concentrations, 24-hour urinary free cortisol/cortisone, plasma corticosteroid-binding globulin (CBG), low dose (1 mcg IV) ACTH stimulation tests with measures of plasma total and free cortisol concentrations were performed.

Results: Mean arterial pressure fell by 6 mmHg after 7.7 kg weight loss. Salt loading elevated day time blood pressure by 6/3 mmHg which was not altered by weight loss. Plasma aldosterone and renin levels fell with weight loss (aldosterone: 853 ± 156 to 635 ± 73 pmol/L ; renin: 35.4 ± 7 to 24 ± 3 mU/L $P<0.05$). HPA axis measures were not affected by weight loss; there was no change in the plasma total and free cortisol responses to cosyntropin nor did plasma CBG levels change. The 11 beta hydroxysteroid dehydrogenase 1 (11β-HSD1) activity, represented by the ratio of urinary free cortisol to cortisone, and the 24-hour urine free cortisol levels were also unchanged.

Conclusions: Short-term, moderate weight loss was associated with a small reduction in blood pressure and reduced levels of aldosterone and renin. The blood pressure elevating effect of a salt load was not altered. Cortisol secretion or metabolism were unchanged. These findings suggest that aldosterone may have an important role in the BP fall with weight loss via a renin mediated mechanism, perhaps involving renal sympathetic tone.

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PREVALENCE OF VITAMIN D DEFICIENCY IN PATIENTS UNDERGOING PRIMARY ELECTIVE KNEE AND HIP ARTHROPLASTY.

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Vitamin D (25OHD) deficiency remains highly prevalent in western countries including Australia (1). Groups at risk include nursing home residents, darkly-pigmented people and veiled and breast-feeding mothers and their offspring. Osteoarthritis (OA) is the most prevalent form of arthritis causing restricted mobility and hence potentially limits sunlight exposure. This study was undertaken to determine the prevalence of 25OHD deficiency in patients undergoing elective hip and knee arthroplasty for OA. 192 consecutive patients (59M) aged 15-96yr (median 66yr) referred to one surgeon for arthroplasty over 29 months were studied. Those already taking vitamin D supplements and those without OA were excluded. Concentrations of 25OHD were measured by competitive protein binding (Nichols Advantage Specialty System) and classified as no (25OHD > 65 nmol/L), borderline (50.1-65), mild (25.1-50), moderate (12.5-25) or severe (<12.5) deficiency (2). The range was 17-201 nmol/L with moderate deficiency in 5.2% (n=10, all female), mild in 34.9% (52F, 15M), and borderline in 20.3% (25F, 14M). Mild or moderate deficiency was more prevalent in females (F: 62/133, 46.6% vs M: 15/59, 25.4%, chi-square = 7.64, p=0.01). Although 16/27 (59.3%) of females tested in winter had mild or moderate deficiency and only 1/12 (8.3%) of males tested in autumn had mild deficiency, overall there was no significant seasonal variation (chi-square = 6.2, p=NS). Mean (±S.D.) seasonal 25OHD concentrations were: summer 58.7±32.1 (n=43) nmol/L, autumn 64.3±25.8 (54), winter 58.5±21.1 (46), and spring 59.4±25.4 (49). 25OHD concentrations did not correlate with age, height, weight or BMI.

In this relatively high socioeconomic group, patients with severe OA (especially females) are at significant risk of 25OHD deficiency. Since this may cause suboptimal new bone quality (in non-cemented prostheses)(3) and reduced muscle tone during rehabilitation which may contribute to risk of falls, 25OHD deficiency should be excluded prior to surgery.

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ANDROGEN MEDIATED ERYTHROPOIESIS OCCURS VIA CLASSICAL ANDROGEN RECEPTOR SIGNALLING IN MALE MICE

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Androgens are known to increase erythropoiesis however the mechanisms remain unclear. Both the use and abuse of androgens have been shown to increase haematocrit and in some cases to higher than normal levels while anaemia has been reported following treatment with various forms of androgen blockade. Many of the clinical and in vivo and in vitro studies to establish androgen effects on erythropoiesis are old (>20 years) and have reported conflicting data.

We now have improved research tools to investigate the mechanisms of androgen mediated erythropoiesis. We have generated a global androgen receptor knockout mouse (ARKO). In this mouse model classical signalling of the AR is disrupted by deletion of exon 3 which encodes the second zinc finger of the DNA binding domain (Notini, Davey et al. 2005).

Red blood cell (rbc) parameters measured in the blood of 9 to 10 week old male ARKO mice were not significantly different from their male wildtype (wt) littermates. ARKO (n=9): rbc 8.58 (mean) ± 0.14 (SE), Haematocrit (Hct) 45 ± 1.3, Haemoglobin (Hb) 137 ± 1.1; wt (n=3): rbc 8.50 ± 0.11, Hct 44 ± 1.2, Hb 135 ± 1.8. These findings are unsurprising given that there are no reports of anaemia in individuals with androgen insensitivity syndrome.

We have also treated 3 to 4 week old male ARKO mice and their male wt littermates with subcutaneous Silastic implants containing testosterone for a 6 week period. Blood, bone marrow and spleen were collected for differential analysis. Our preliminary results show that the red blood cell count is increased by 6% in response to testosterone in male wt mice and NOT male ARKO mice. Wt (plus testosterone) (n=5): rbc 9.00 ± 0.12 vs wt (no treatment) (n=3): rbc 8.50 ± 0.11, $p<0.05$.

In conclusion, we have demonstrated that the classical signalling pathway of the AR is required for androgen mediated erythropoiesis.

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OESTROGEN REGULATION OF THE LIVER RECEPTOR HOMOLOGUE (LRH-1) WITHIN TESTICULAR CELLS.

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The orphan nuclear receptor LRH-1 is expressed in Leydig cells, pachytene spermatocytes and round spermatids, which is similar to the expression of aromatase. We have previously shown that LRH-1 can regulate aromatase expression in these cells by directly binding to the gonadal-type promoter of the CYP19 gene (promoter II) and stimulating transcription. Little is known regarding the regulation of LRH-1; however, recently a role for estrogen receptor alpha (ERalpha) in stimulating LRH-1 expression in breast cancer cells was proposed[(1)]. We therefore hypothesised that aromatase may maintain its own expression in the testis by inducing ERs to activate LRH-1 expression, which in turn increases aromatase promoter II activity. To begin to address this we have used a transgenic mouse that over-expresses human aromatase[(2)] (Arom+). Compared to wild type mice, Arom+ mice displayed a 553% increase in LRH-1 protein expression in the whole testis as determined by Western analysis. Total ER protein levels were also over-expressed in the Arom+ mice by 168%. Immunohistochemical analysis demonstrated that LRH-1 is co-localised with ERalpha in Leydig cells, and with ERbeta in Leydig cells, pachytene spermatocytes and round spermatids in both wild type and Arom+ mice. We are currently assessing whether oestrogen can directly stimulate LRH-1 expression in appropriate cell lines to determine the pathway of this apparent positive feedback mechanism. Our current work therefore indicates that LRH-1 has important roles in estrogen production and regulation in the testis, which may in turn have implications for testicular development, fertility and tumorogenesis.

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'GAIN OF FUNCTION' ANALYSIS IN SKELETAL MUSCLE CELLS SUGGESTS RETINOIC ACID RECEPTOR RELATED ORPHAN RECEPTOR GAMMA CONTROLS METABOLIC GENE EXPRESSION.

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Nuclear Hormone Receptors (NRs) have been demonstrated to regulate metabolism in a cell/organ specific manner. The NR1F (Retinoic acid receptor related Orphan Receptors - RORs) subgroup includes three members: RORα, RORβ and RORγ. The staggerer mice carry a deletion in the RORα gene and display lowered plasma apoA-I/-II, apoC-III, decreased plasma high density lipoprotein cholesterol and triglycerides, develop hypo-α-lipoproteinemia and atherosclerosis. Previously we have shown that, RORα regulates the expression of genes involved in lipid homeostasis in skeletal muscle cells. However, RORγ is also abundantly expressed in skeletal muscle, a major mass peripheral tissue that accounts for 40% of total body mass and energy expenditure. This lean tissue is a primary site of glucose and lipid utilization. Consequently, we utilized gain and loss of function studies in skeletal muscle cells to understand the regulatory role of RORγ in muscle cells. Exogenous dominant negative RORγ expression in skeletal muscle cells specifically represses the endogenous levels of RORγ mRNA, however 'loss of function' did not show a clear phenotype. Interestingly exogenous VP16-RORγ expression (i.e. gain of function) in skeletal muscle cells enhances the endogenous levels of RORα and RORγ mRNA expression. In addition, we observed enhanced expression of many genes involved in lipid homeostasis. This study implicates RORγ in the control of fatty acid utilization in skeletal muscle cells.

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REGULATION OF BODY COMPOSITION DEVELOPMENT BY THE SKI PROTO-ONCOGENE *IN VIVO*

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Introduction: Body composition (i.e. the amount and proportion of body fat, lean tissue and bone) is regulated by a variety of metabolic and hormonal processes and is an important determinant of general health. The Ski proto-oncogene, is a negative regulator of TGF-β signalling. Ski overexpression in a transgenic mouse model leads to a marked increase in muscle and decrease in fat mass. The aim of this study was to investigate the molecular mechanisms of Ski mediated regulation of body composition.

Methods: Growth analysis was conducted on c-Ski Tg mice and their wild-type littermates. At 15 weeks a detailed body composition (BC) analysis was performed using dual-energy X-ray absorptiometry. Gastrocnemius muscles were harvested from 12 month old male mice for gene expression analysis by quantitative real-time PCR.

Results: Ski Tg mice gained more weight between 4 and 15 weeks of age, than their wild-type littermates. At 15 weeks BC analysis of male Tg mice showed both increased lean body mass (26.9±2.2g vs 20.8±1.0g) and reduced total fat mass (2.5±0.5g vs 3.1±0.2g). Expression analysis, relative to wild-type mice, of key metabolic genes revealed a 2-fold decrease in myostatin ($P<0.001$), a negative regulator of muscle growth, and a 10-fold decrease in SREBP-1c ($P<0.001$), a key transcriptional activator of lipogenesis. Transactivation assays revealed that Ski represses activity of the SREBP1c promoter. Expression of downstream targets of SREBP-1c, FAS and SCD-1, were downregulated by 3-fold ($P<0.005$). Attenuation of expression of the glucocorticoid receptor, PPARγ and several orphan NRs involved in metabolism was observed.

Conclusion: The skeletal muscle of c-Ski Tg mice have significant alterations in expression of several muscle and lipogenic regulatory genes. This suggests Ski acts as a major regulator of factors that control body composition and hence risk for obesity and metabolic disease.

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NR3B3 (ERRγ) CONTROLS PATHWAYS THAT REGULATE MUSCLE MASS AND ADIPOSITY IN SKELETAL MUSCLE CELLS.

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Nuclear hormone receptors (NRs) are ligand dependent DNA binding proteins, that translate physiological and nutritional signals into gene regulation. The estrogen receptor-related receptors (ERR) are orphan members of the steroid and NR gene superfamily. The ERR subfamily is comprised of three members ERRα (NR3B1), ERRβ (NR3B2) and ERRγ (NR3B3). ERRα has been demonstrated to control lipid, glucose and energy homeostasis, in an organ specific manner. ERRγ is abundantly expressed in tissues with onerous energy demands such as skeletal muscle (heart, kidney and brown adipose) that depend on mitochondrial fatty acid oxidation for energy. ERRγ is more closely related to ERRβ in its sequence than ERRα, however, its pattern of expression is similar to that of ERRα. PGC-1 is a critical coactivator of ERRα, and has also been demonstrated to interact with ERRγ and serves as a key regulator of mitochondrial biogenesis and mitochondrial oxidative metabolism.

Skeletal muscle, a major mass tissue accounts for ~40% of the total body mass and energy expenditure, and is a major site of fatty acid and glucose oxidation. The obscure role of this orphan NR in this peripheral lean tissue prompted us to investigate the ERRγ function in the regulation of genetic programs that control lipid utilization in skeletal muscle cells. We demonstrate that ERRγ is dramatically induced during in vitro skeletal myogenesis. Moreover, we show that the recently described ERRγ agonist and antagonist, increase and decrease GAL4-ERRγ activity in skeletal muscle cells, respectively, in a dose dependent manner. Furthermore, we demonstrate that ERRγ, a constitutively active orphan NR interacts and recruits coactivators in an agonist independent manner. In the in vitro cell culture model, treatment of cells with ERRγ agonist and antagonists leads to changes in gene expression involved in the regulation of muscle mass and adiposity. In conclusion, NR3B3/ERRγ regulates pathways that control muscle mass, adiposity and lipid utilization in skeletal muscle.

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INCREASED INTRAMYOCELLULAR LIPID CAUSES SKELETAL MUSCLE INSULIN RESISTANCE IN THE YOUNG ADULT GUINEA PIG OF LOW BIRTHWEIGHT

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Restricted fetal growth is characterised by insulin resistance of glucose uptake and utilisation in skeletal muscle in the adult, but the underlying mechanisms responsible are poorly understood. Some known risk factors for insulin resistance act via impaired 5' AMP activated protein kinase (AMPK)/ malonyl CoA fuel sensing and activity, increasing intramyocellular lipids, which impair insulin signalling and its targets. We therefore hypothesized that fetal growth restriction would cause skeletal muscle insulin resistance and increase intramyocellular lipids in the young adult guinea pig, and that activation of AMPK by 5-aminoimidazole 4-carboxamine-riboside (AICAR) to lower intramyocellular lipids would normalise this. Young adult guinea pigs of varying size at birth (n=16) underwent hyperinsulinaemic euglycaemic clamps (HEC) with a bolus of D-2-[1-^{14}C]-deoxyglucose to measure whole body and skeletal muscle insulin sensitivity. Intramyocellular lipid in vastus lateralis and biceps femoris was also quantified. Additional guinea pigs (n=9) were treated with AICAR (60mg/kg sc every 2 days) then HEC carried out. Intramyocellular lipid in the vastus lateralis and biceps femoris generally correlated negatively with birthweight in controls, but positively with birthweight in AICAR treated animals. In particular, low birthweight female guinea pigs exhibit a decrease in intramyocellular lipid in the biceps femoris following AICAR. Insulin sensitivity of glucose uptake and phosphorylation, incorporation into glycogen in skeletal muscle decreased with birthweight in controls, but this was abolished by AICAR. AICAR also increased whole body insulin sensitivity of young adult guinea pigs of low birthweight (+50%) to that seen in high birthweight animals. Therefore AMPK activation by AICAR normalised intramyocellular lipid and insulin sensitivity, suggesting that dysregulated lipid metabolism and accumulation of inhibitory lipid species caused the prenatally induced insulin resistance.

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ANDROGEN REGULATION OF SATELLITE CELL FUNCTION IN VITRO

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Androgens increase the size and strength of muscle in humans. Satellite cells (quiescent myoblasts) are the major source for muscle growth and regeneration. The androgen receptor (AR) has been found in satellite cells. However, the mechanism by which androgens regulate satellite cell function remains unclear. The present study is to investigate the effects of androgen regulation of myoblasts in vitro. Firstly, due to the low level of the AR expression in endogenous C2C12 cells (a mouse myoblast cell line), C2C12 cells overexpressing the AR cDNA driven by the SV40 promoter or control plasmids were generated by stable transfection. Secondly, qualitative determination of the level of the AR protein in C2C12 cells overexpressing the AR cDNA was performed using Western analysis. The cell line demonstrating the highest level of the AR protein compared to the endogenous C2C12 cells was chosen for further experiments. Transfected cells were cultured in charcoal stripped fetal calf serum (CS-FCS) with the addition of either testosterone or dihydrotestosterone (DHT) at concentrations ranging from 10-9 to 10-6 M every 24 hours for up to 3 days. The MTT assay was used to quantitate cell proliferation. No significant effect of androgens on proliferation of transfected C2C12 cells was observed. Differentiation of myoblasts into myotubes is indispensable for myoblast contributing to myofiber formation. The differentiation of C2C12 cells was induced with 3% CS-horse serum with administration of 10-7M DHT or vehicle. Creatine kinase, which is produced by differentiated myoblasts, is being used to quantitate the effect of androgens on myoblast differentiation. Investigation of the change of the AR mRNA signal and other potential androgen responsive target genes in transfected cells will be conducted by using real-time PCR to further explore roles of androgens in modulation of satellite cell function.

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METASTATIC MACROPROLACTINOMA IN MULTIPLE ENDOCRINE NEOPLASIA TYPE 1 (MEN-1) MIMICKING MENINGIOMA IN CERVICAL CORD

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Prolactinomas are common, but metastatic prolactinomas rare. We report a 47 year-old man with MEN-1, and a metastatic prolactinoma masquerading as a cervical meningioma. He has an IVS2-3 mutation (splice site C→G substitution[1]). A 7mm microprolactinoma was diagnosed on computerised tomography (CT) pituitary in 1990. Prolactin was 7355 mU/L. He was lost to follow up. Three years later prolactin was 26,833 mU/L and the tumour was compressing the optic chiasm. On bromocriptine for 1 year prolactin fell to 6545mU/L and the tumour shrank. Prolactin increased to 18,998 mU/L over 2 years, and the tumour grew to the superior aspect of the right internal carotid artery, depressed the floor of the fossa into the right sphenoid sinus, and bowed the optic chiasm. Trans-sphenoidal hypophysectomy was performed. After three years of loss to follow-up, the tumour had grown to 22 mm diameter. Prolactin was 18,311 mU/L. Cabergoline, 2mg / week, did not shrink the tumour, which could not be excised trans-sphenoidally. Incomplete excision was performed by craniotomy and 45Gy radiotherapy given. Magnetic resonance imaging (MRI) showed no apparent residual tumour. Six years later he developed neck pain initially thought to be due to cervical spondylosis, but prolactin was 98,680 mU/L and MRI showed masses in the spinal cord, cerebello-pontine angle and the left side of the foramen magnum. The cervical tumour was initially misdiagnosed as a meningioma but resection confirmed prolactinoma. This spinal lesion was extradural, lobulated, slightly hyperintense to the spinal cord on T1-weighted images and showed moderate and uniform enhancement after intravenous contrast, all commonly seen with meningioma. In the posterior fossa there was enhancement of the tentorium, consistent with a dural tumour tail of meningioma.

In patients with macroprolactinoma, particularly if refractory to dopaminergic therapy, any neurological symptoms or signs may be related, even if extra-cranial.

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ABLATIVE RADIOIODINE THERAPY IN A CASE OF CONCURRENT THYROID CARCINOMA, GRAVES' DISEASE WITH ASSOCIATED OPHTHALMOPATHY AND JUVENILE ONSET GLAUCOMA

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Radioiodine therapy is a well established treatment for differentiated thyroid carcinoma and Graves' hyperthyroidism. However, radioiodine therapy has been associated with exacerbation of Graves' ophthalmopathy, which can be ameliorated by the administration of corticosteroids. We hereby report the case of a 42 year-old man with a background of Juvenile Onset Glaucoma who presented with severe Graves' hyperthyroidism (FT4 >77 pmol/L), Graves' ophthalmopathy and cervical lymphadenopathy, with FNA cytology indicating metastatic papillary carcinoma. He was rendered euthyroid with propylthiouracil and underwent a total thyroidectomy and right modified radical neck dissection. Histopathology confirmed the presence of papillary carcinoma with metastatic disease to ten lymph nodes. Four weeks after his thyroidectomy, he received his initial dose of ablative radioiodine. In light of his Graves' ophthalmopathy, he was administered prophylactic corticosteroids. His treatment was complicated by a worsening of his Graves' ophthalmopathy in addition to acutely rising intra-ocular pressures. After an urgent ophthalmology review, his glaucoma medications were altered, corticosteroids were continued and he had a subsequent recovery from his eye symptoms. The concurrent presence of all of these conditions in a patient is rarely encountered and this case demonstrates the challenges faced by clinicians when weighing up the benefits of radioiodine therapy, the risks of a deterioration of Graves' associated ophthalmopathy and the potential side effects when prophylactic corticosteroid are utilised.

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PITUICYTOMA: AN UNUSUAL PRIMARY TUMOR OF THE PITUITARY WITH CHARACTERISTIC MRI AND HISTOPATHOLOGICAL FINDINGS

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Mr P.W., a 43 yo single male, identical twin, presented with a 2 year history of lethargy and declining physical strength. He had noted 15 months of headaches and occasional visual disturbance on a background of several years of gradual loss of body hair and a reduced libido. Past medical history included recurrent finger infections, amoebic dysentery and surgery for an undescended testicle at age 6. He was on no regular medications. Examination revealed features of hypopituitarism and in particular hypogonadism. A remarkable feature of this case was the stark contrast on both history and examination between Mr. PW and his identical twin (photo to be included). Laboratory investigations confirmed secondary hypogonadism and growth hormone deficiency. TSH 2.10 mIU/L fT4 13.3 pmol/L FSH 2.8 U/L LH 1.5 U/L Prolactin 15.3 mcg/L Testosterone 1.5 nmol/L Cortisol (am) 382 nmol/L HCG < 2 U/L IGF-1 12.4 nmol/L alpha-FP 5 mcg/L MRI of the pituitary showed a "typical" round, lobulated, contrast-enhancing 16mm x 29 mm suprasellar mass . Stereotactic guided needle biopsy of the lesion was consistent with the diagnosis of pituicytoma. This was confirmed after excision of the mass via a craniotomy. Postoperative recovery was uneventful. Mr PW continues on pituitary replacement therapy. A literature review highlighting the histology, imaging features and clinical experience of this rare primary tumor of the pituitary will be presented. Questions for the audience: 1) What is the differential diagnosis? 2) How long has the tumor been present? 3) What is the risk to his identical twin? 4) What are the implications of a suprasellar lesion and its treatment? 5) What is the prognosis - endocrine and tumour related? 6) Should adjuvant treatment be used?

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METABOLIC REHABILITATION: A NEW APPROACH TO MANAGING OBESE PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Aim: We evaluated a novel ambulatory care clinic treating obese adults with type 2 diabetes mellitus (T2DM) using weight management as the primary intervention.

Patients and methods: Patients able to undergo an intensive lifestyle program were eligible. The exercise program consisted of a total of 330 minutes of weekly physical activity that included at least three supervised 1-hour exercise classes. Lifestyle intervention employed dietary, psychological and medical review. Specific obesity therapy

(Sibutramine, Orlistat or meal replacements) was used as indicated and weight neutral therapeutic agents were used in preference to weight inducing anti-diabetic agents.

Results: 37 consecutive adults were eligible; over 12 months of follow-up, 11 dropped out. 26 patients (17 female) with a mean age of 61 (44-83) years and a body mass index of 36.0 (30.0-45.5) kg/m^2 were analysed. The average duration of T2DM was 9.0 years. All patients had the National Cholesterol Education Panel defined metabolic syndrome and 62% were managed by an Endocrinologist prior to clinic referral. In addition, there was a reduction or cessation of at least one metabolic/cardiovascular pharmaceutical agent among all participants.

	Mean ± SD		Change (%)
	Baseline	12 months	
Weight (kg)	96.3 ±15.7	86.9 ± 15.5	- 9.8^
Waist circumference (cm)	112.0 ± 11.3	99.0 ± 11.6	- 11.6^
HbA1c (%)	8.1 ± 1.6	6.9 ± 1.0	- 14.8^
Systolic Blood Pressure (mmHg)	141 ± 15.7	125 ± 9.0	- 11.3^
Diastolic Blood Pressure (mmHg)	81 ± 7.6	73 ± 4.5	- 9.9^
Triglycerides (mmol/L)	1.9 ± 0.9	1.5 ± 0.6	- 21.1^
HDL-Cholesterol (mmol/L)	1.2 ± 0.3	1.4 ± 0.4	+ 16.7*

^p<0.001; *p=0.01

Conclusion: An intensive lifestyle program using a weight management approach as the primary intervention in obese patients with sub-optimally controlled T2DM achieves significant improvements in weight, glycaemic control, blood pressure and lipids while decreasing pharmacotherapy use.

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EVIDENCE FOR FUNCTIONAL EXPRESSION OF VASCULAR AT2 RECEPTORS IN PATIENTS WITH INSULIN RESISTANCE

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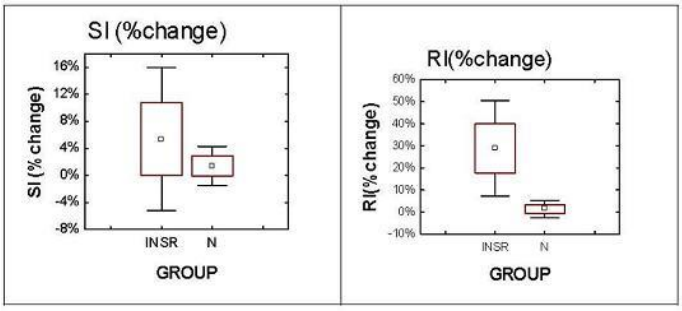
Angiotensin II type 2 receptors (AT2 receptors) are believed to become over-expressed in response to cardiovascular damage and to mediate beneficial effects of vasodilation and suppression of vascular proliferation. However, it is unknown whether AT2 receptors are functionally expressed in patients with insulin resistance (IR). We studied nine subjects with IR (mean age 31±7 yrs, BMI 28.6 ± 4.1 kg/m2, mean cholesterol level 4.7 ± 0.6 mmol/L, mean HOMA-IR mean 2.6 ± 1.0) and nine age- and sex-matched normal subjects (mean age 31±9 yrs, BMI 23.5 ± 2.2 kg/m2, mean cholesterol level 4.1 ± 0.6 mmol/L). All subjects were normotensive, on no medication and were non-smokers. The subjects received a 3 minute infusion of PD123319 (a highly selective AT2 receptor antagonist). Arterial stiffness indices (SI: stiffness index and RI: reflective index), were measured using digital photoplethysmography (Pulse Trace, Micro Medical, Gillingham, Kent, UK) and haemodynamic measurements (CI: cardiac index, SVRI : systemic vascular resistance index and ZI: stroke index) by transthoracic bioimpedance (Cardiodynamics International Corporation, San Diego, CA. BioZ system) at the end of the infusion.



RI increased significantly (p = 0.007) in patients with IR compared to controls (figure-% change RI) which was not accompanied by any significant changes in SI, SBP , DBP, SVRI , CI, ZI.

These results suggest the expression of AT2 receptors in small vessels that determine the inflection of the digital volume pulse wave, and imply the functional expression of vascular AT2 receptors in patients with IR, possibly as an indicator of early vascular damage.

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ELECTRONIC AUDIT IN PRIVATE ENDOCRINE PRACTICE OF EFFICACY & TOLERABILITY OF ROSIGLITAZONE IN TRIPLE ORAL THERAPY

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Background: The pharmacotherapeutic options for patients with Type 2 diabetes and failed dual oral hypoglycaemic therapy have recently been expanded to include addition of rosiglitazone as an alternative to initiation of insulin. The efficacy and tolerability of rosiglitazone in this therapeutic setting has not previously been studied outside of the teaching hospital environment. Modern, low cost clinical software now makes road testing of such drugs feasible in private practice.

Design: Retrospective audit of efficacy and tolerability of rosiglitazone in triple oral therapy – analysis to 6 months.

Method: Audit4 clinical software (S4S Pty Ltd, Australia) was used to identify subjects, and to source and sort their clinical data (demographics, weight, HbA1c, lipids, liver function, drug initiation and cessation dates and cessation reason).

Subjects: Search function identified 101 patients initiated on rosiglitazone in addition to current treatment with metformin and sulfonylurea: 51 male, 50 female, mean age (SD) range 60y (11.1) 32-84, with baseline HbA1c 9.38% (1.26).

Findings: Of the 101 rosiglitazone initiations, 15% patients had ceased within 6 months, 3% because of lack of efficacy, 10% because of adverse reaction, most commonly unacceptable weight gain (2%) and oedema (3%). Of those patients still on rosiglitazone at 6 months, HbA1c fell by mean (s.e) 1.6 % (0.3) and weight increased 2.6 kg (0.5). Compared to baseline, significant increases ($p<0.05$) were found in mean (s.e) values of total cholesterol +0.39mM (0.12) and LDL-cholesterol +0.43mM (0.13), and significant falls in ALP -19.7 U (3.0) and GGT – 13.0 U (2.6).

Conclusions:

1. The findings in community practice support the efficacy and safety of initiation of rosiglitazone as the initial step-up from dual oral therapy, prior to insulin therapy.

2. Appropriate clinical audit software enables clinicians in private practice to analyse their own data and provide a rational basis for management decisions.

(Acknowledgement: author thanks GSK for financial support for this study)

HbA1c (%) Change	count	% patients		Weight (kg) change	count	percentage
< 0.5	9	23.7%		< 2kg	18	46.2%
0.5 - 1.0	4	10.5%		2kg - 5kg	13	33.3%
1.0 - 1.5	4	10.5%		5kg - 10kg	8	20.5%
1.5 - 2.0	9	23.7%		> 10kg	0	0.0%
> 2.0	12	31.6%				

Table 1: Distribution of change in HbA1c and weight at 6 months post initiation rosiglitazone

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CLINICAL PROFILE OF PATIENTS WITH MICROALBUMINURIA

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The appearance of microalbuminuria in a diabetic patient predicts the development of diabetic nephropathy and cardiovascular disease. In patients with type 2 diabetes and microalbuminuria, the Steno-2 study has shown 50% reduction in the risk of cardiovascular and microvascular events with intensified intervention aimed at multiple risk factors.

We compared the clinical profile of our patients with microalbuminuria with patients in the Steno standard and Steno intensive sub groups. We then used the UKPDS risk engine to calculated the 10 year cardiovascular risk profile of our patients.

One thousand three hundred and thirty four patients with type 2 diabetes attended the diabetes assessment clinic at The Queen Elizabeth Hospital between January 2004 and March 2006. Two hundred and thirteen were detected to have microalbuminuria. This group comprised of 60% males vs 70% and 79% in the Steno standard and the Steno intensive subgroups. Our patients were older (61+/-13 vs 55+/-7.2 yrs), had shorter duration of diabetes (1.3 vs 6 and 5.5 yrs), had lower HbA1c (7+/-1.5 vs 8.8+/-1.7 and 8.4+/-1.6 %) and lower serum cholesterol levels (4.6+/-1 vs 5.8 +/-1.3 and 5.4 +/-1.1 mmol/L), their mean systolic blood pressure was (136+/-22 vs 149+/-19 and 146+/-20 mmHg). Our patients comprised of 20% smokers vs 34% and 40% in the other two groups. There was a higher prevalence of stroke (9% vs 3% and 2%) and peripheral vascular disease (7% vs 2.5% and 1%) in our patients. The prevalence of ischaemic heart disease was lower (10% vs 29% and 23%) and that of retinopathy was similar between the three groups (26%).

By lowering HbA1c to < 7%, total cholesterol to <4 mmol/L and systolic blood pressure to 130 mmHg, the absolute 10 years risk of coronary heart disease in our patients can be reduced from 17.7% to 11.2%.

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SCREENING FOR PHAEOCHROMOCYTOMA IN RENAL FAILURE

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35 male was referred to the Endocrinology outpatient clinic for further investigation of severe hypertension after a review by his GP in Dec 2005 when he presented with the predominant symptom of fatigue. Investigations included 24hr urinary catecholamine collection and renal function tests. His had a past history of a solitary left kidney with reflux and evidence of proteinuria since 1990, but had been lost to follow up.

He was found to have significant renal impairment with a Creatinine of 615umol/L, the patient had not noticed a decreased urine output. 24 hour urinary catecholamines were positive with a noradrenaline level of 1729nmol/day and dopamine of 6.1

Further investigations by the renal unit at a tertiary hospital included plasma metanephrines; these were elevated with a normetadrenaline level of 910pmol/L (<780) and metadrenaline of 320pmol/L (<300). Imaging, including a CT abdomen and a MIBG scan were negative.

The patient subsequently commenced haemodialysis and once stabilised had his first Endocrinology outpatient visit, querying the possibility of phaeochromocytoma accountingin part for his hypertension.

BP readings have remained around 210/100 despite therapy with four agents.

Further plasma metanephrines pre and post dialysis are being processed.

Questions raised include:

How to interpret screening investigations for phaeochromocytoma (1,2) in the setting of renal failure, in particular given the less reliable method of 24 hour urine collection?

If plasma metanephrines and imaging are used in patients with renal failure (3), how should the plasma metanephrines in a patient on haemodialysis be interpreted and what impact does haemodialysis have on these results and thus is the timing of collection relative to haemodialysis important?

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GRAVES' DISEASE IN PREGNANCY: WHEN MORE THAN JOY ALONE CAN SET YOUR HEART RACING

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Management of hyperthyroidism in pregnancy is challenging as one has to consider both mother and baby.

Case 1

A 35 year old woman with a prior history of Graves' disease developed a post-partum exacerbation of thyrotoxicosis after her first pregnancy and required antithyroid medication. Whilst on carbimazole 10mg daily, she fell pregnant again with thyroid function showing thyroid stimulating hormone (TSH) 0.01mU/L, free T4 39.1pmol/L, free T3 8.9pmol/L with positive thyroid receptor antibodies (TRAb) 8.7U/L (reference range 0-1). She was thyrotoxic with heat intolerance, palpitations and anxiety. Thyroid hormone levels increased during the first trimester (fT4 36.7pmol/L, fT3 17.1pmol/L) and carbimazole was increased to 10mg bd. During antenatal surveillance, a major congenital heart disease was detected in the fetus. She is currently in the third trimester, clinically stable but remains mildly thyrotoxic (TSH

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MULTIPLE INSUFFICIENCY FRACTURES OF THE PELVIS AND FEMORA IN A POST-MENOPAUSAL WOMAN ON ALENDRONATE THERAPY

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Insufficiency fractures are increasingly recognized as a clinical entity in the aging population. Likely to be the result of a discrepancy between physiological stress and skeletal strength, they occur most commonly in the pelvic girdle, the sacrum, the tibia and the femoral neck. Insufficiency fractures of the femoral diaphyses are rare, with only few reported cases. The strongest associations exist with untreated osteoporosis. We describe an unusual case of multiple insufficiency fractures in a 73-year-old Chinese woman who presented with a 12 months history of bilateral groin pain and difficulty with walking in the absence of trauma, diagnosed 18 months [Fig. 1.1 and 1.2] following the commencement of alendronate, highlighting the challenge in the management of this condition. Presentation is non-specific; plain radiographs are commonly normal initially, with fracture lines, fracture callus or osteocondensation visible in only about 60% of cases. Scintigraphy is more sensitive in the detection of such fractures. Pathogenesis of insufficiency fractures is poorly understood. Possible mechanisms may not be accountable solely by low bone mineral density, and may be attributable to over-suppression of bone turnover during long-term use of bisphosphonates, microcracks accumulation and microarchitectural distortion. This case aims to increase the recognition of insufficiency fractures, especially in patients with risk factors and atypical pain not accountable by changes on plain radiography, and to promote utilization of more sensitive imaging modalities for investigation. Prompt referral to physiotherapy to rectify an abnormal gait may be a crucial yet neglected step in the prevention of insufficiency fractures.

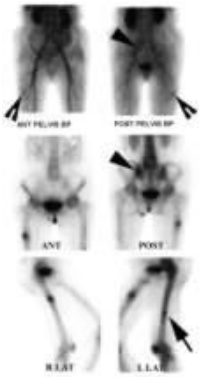

Figure 1.1 Hyperemia in the lateral cortex of the diaphysis of the right femur (open arrowhead), and the left sacroiliac joint (solid arrowhead). Delayed images show uptake in the lateral cortex of the right femoral diaphysis (open arrowhead).

Figure 1.2 A transverse breach of the thickened lateral cortex of the right femur (arrowhead) corresponding to the scintigraphic site of fracture. A thickened lateral cortex is evident in the left femur (arrow), with no discrete fracture line.

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THE PROSTATE-SPECIFIC ANTIGEN (*PSA*) POLYMORPHISM (G-158A) ALTERS INTERACTION OF ANDROGEN RESPONSE ELEMENT 1 WITH THE ANDROGEN RECEPTOR

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Prostate cancer accounts for approximately 10% of all male cancers and is the fourth leading cause of cancer deaths in men. Although not well understood, it is well established that androgens play an important role in the earlier stages of prostate cancer aetiology. The prostate specific antigen or kallikrein 3 (*PSA/KLK3*) gene is primarily expressed in the prostate and has been shown to process various factors that are important in prostate (patho)-physiology, suggesting an active role for PSA in prostate cancer progression. In the prostate, *PSA* is regulated by androgens with three well-described androgen response elements (AREs) identified in the *PSA* promoter. Interestingly, a polymorphism that results in a G to A transition (G-158A) is located within the second canonical half-site of *PSA* AREI. Some epidemiological studies suggest that G-158A is associated with risk of developing prostate cancer. We have therefore, investigated the functional significance of the G-158A polymorphism in prostate cancer using *in silico* and *in vitro* analyses. We have found that the *PSA* AREI alleles bind with a two-fold difference with the AR-DBD, which was further confirmed in limited proteolysis experiments. Molecular modelling for binding of the variant AREI alleles with the AR-DBD suggest that these differences may be conferred by the introduction of two extra hydrogen bonds for the A allele at the -160 position of AREI with Arg_{568} of the androgen receptor. Furthermore, reporter assays using three-tandem copies for each *PSA* AREI allele demonstrated that the G-158A polymorphism also alters androgen induced transactivation of the *PSA* promoter in two different prostate cancer cells. In conclusion we have shown that the G-158A polymorphism alters interaction of the AREI with the androgen receptor, which may account for the observed differential expression of PSA in prostate cancer, as well as the association of this polymorphism with prostate cancer risk.

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IGF-I:IGFBP:VN COMPLEX ENHANCED BREAST CANCER CELL MIGRATION INVOLVES BOTH VN-BINDING INTEGRINS AND THE IGF-1R THROUGH ACTIVATION OF THE AKT/PI3-K SIGNALLING PATHWAY.

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We previously reported that IGF-I can bind to the extra-cellular matrix protein vitronectin (VN), via the involvement of select IGFBPs. As both IGF-I and VN have established roles in promoting tumour cell dissemination we wished to investigate the functional consequences of the interaction of IGF-I, IGFBPs and VN in the model MCF-7 breast cancer cell line. Substrate-bound IGF-I:IGFBP:VN complexes stimulated synergistic increases in cellular migration above that induced by the individual components alone. This effect was also seen in non-tumourgenic MCF-10A mammary epithelial cells. Furthermore, studies using IGF-I analogs determined this stimulation to be dependent upon both ternary complex formation and the IGF-1R. The synergistic effects on cellular migration were abolished upon incubation of cells with function blocking antibodies directed at VN-binding integrins, in particular αvβ5, and the IGF-1R. IGF-I:IGFBP:VN complexes stimulated transient activation of the ERK/MAPK signalling pathway, while also producing a sustained activation of the AKT/PI3-K pathway. However, the AKT/PI3-K pathway was only activated when all the components of the complex were present. Experiments using pharmacological inhibitors of the PI3-K/AKT pathway, have shown that activation of this pathway is vital for IGF-I:IGFBP:VN complex stimulated cell migration, whereas the ERK/MAPK pathway was not. To confirm these results we are currently over-expressing wild-type, dominant negative and constitutively active-AKT to determine the effects on IGF-I:IGFBP:VN stimulated migration. Furthermore, microarray experiments have also been undertaken to identify candidate genes involved in enhanced cell migration. Results from this project will contribute valuable information regarding tumour cell metastasis and result in the development of improved therapeutics for the treatment of breast cancer and other carcinomas.

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EXPRESSION OF COMPONENTS OF THE GHRELIN/GROWTH HORMONE SECRETAGOGUE RECEPTOR AXIS IN BREAST CANCER CELL LINES AND IN BREAST CANCER HISTOPATHOLOGICAL SPECIMENS

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Breast cancer is the most common malignancy in females and is a major cause of death in Western women. While oestrogen and progesterone are clearly implicated in the development of breast cancer, there is increasing evidence that the ghrelin/growth hormone secretagogue receptor (GHSR) axis could be involved. We have previously demonstrated that ghrelin stimulates the growth of prostate cancer cells, which is also a hormone dependent cancer. Ghrelin, a 28 amino acid peptide hormone, mediates numerous physiological functions, including growth hormone release, through the GHSR. In this study, we have demonstrated the expression of ghrelin and its two receptor isoforms (GHSR1a and 1b) in the MDA-MB231, MDA-MB435, T47D, MCF7 and MCF10A breast cell lines *in vitro* and in histopathological breast cancer tissues. We have also demonstrated that breast cell lines and normal breast tissue express an exon 3 deleted isoform of proghrelin. This novel ghrelin isoform, which we first described in prostate cancer tissue, encodes a novel C terminal peptide. Through real time PCR a 12 fold increase in this exon 3 deleted mRNA isoform in the oestrogen receptor negative breast cancer cell line, MDA-MB-435 has been demonstrated. We have also shown that oestrogen receptor negative breast cancer cells, MDA-MB435 and MDA-MB231 proliferate (up to 40% and 20% above control respectively) in response to ghrelin treatment. Ghrelin may therefore play a role in the autocrine/paracrine stimulation of proliferation in oestrogen receptor negative breast cancers. Through further analysis of this axis, novel therapeutics for these oestrogen receptor negative cancers may be developed.

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NEUROBLASTOMA CELL DIFFERENTIATION BY FIBROBLAST GROWTH FACTOR-2 (FGF-2) INVOLVES REGULATION OF INHIBITOR OF DIFFERENTIATION (ID) GENES AND SUPPRESSOR OF CYTOKINE SIGNALING-2 (SOCS-2)

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An early event in the pathogenesis of neuroblastoma (NB) is the arrested differentiation of neuroblasts. We demonstrated that FGF-2 promotes NB cell differentiation and over-rides the mitogenic action of insulin-like growth factor-I (IGF-I). A number of genes have been implicated in the regulation of neuronal cell proliferation and differentiation, including the transcriptional regulator ID genes and signaling molecules including the SOCS genes. Whether these genes mediate FGF-2 differentiation of NB cells and/or inhibition of IGF-I action is unknown. Therefore we analysed the expression of ID1-3 and SOCS1-3 in SK-N-MC cells cultured in the presence or absence of FGF-2 (50ng/ml) and/or IGF-I (100ng/ml). The TUJ1 and GAP43 markers confirmed neuronal differentiation.

ID1 expression was detected by RT-PCR in untreated and IGF-I induced SK-N-MC cells, but it was decreased by FGF-2. In contrast ID2 was strongly induced by FGF-2, but not in untreated or IGF-I stimulated cells. ID3 mRNA was not affected by treatments.

The FGF-2 signaling modulator SOCS-2, but not SOCS-1/3, was up-regulated by FGF-2. SOCS1-3 were detectable but not regulated in the untreated or IGF-I stimulated cells.

The balance between expression of various ID isoforms is required to maintain normal cellular homeostasis, such that ID1 regulates proliferation and ID2 regulates differentiation. Deviation from this balance might lead to cell transformation and malignancy. Our data provide evidence for unbalanced expression of ID genes in arrested neuroblast differentiation. For the first time we show that FGF-2 reverses this "balance" towards growth arrest (inhibition of ID1) and differentiation (induction of ID2) of NB cells. The induction of SOCS-2 expression by FGF-2 is associated with reduced mitogenic action of IGF-I suggesting inhibition of IGF-IR signaling by SOCS-2, a member of the SOCS family not previously associated with regulation of IGF receptor signaling.

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NEUROBLASTOMA CELL ADAPTATION TO HYPOXIA IS ACHIEVED VIA COMPLEX MODULATION OF GENES ENHANCING CELL SURVIVAL AND METASTASIS.

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Solid tumors, including neuroblastoma (NB), adapt to oxygen and nutrients deprivation by increasing their intra-tumor neovascularisation, leading to invasive/metastatic behaviour. Hypoxia inducible factor-1ά (HIF-1ά), a key regulator of oxygen homeostasis, and its target genes modulate cell survival and growth in cells exposed to hypoxia. In this study SH-SY5Y cells were employed to determine the mechanisms regulating adaptation to hypoxia in NB.

NB cells were cultured in serum free medium in the presence or absence of CoCl2 (100μM, hypoxia mimic) for up to 48 hours.

SH-SY5Y cell number was not affected by CoCl2 treatment, while mitochondrial activity was reduced by about 50%. HIF-1ά protein was detected as early as 30 min post-hypoxia, followed by increases of mRNA for hypoxia responsive genes (ie. erythropoietin, VEGF) at 4 hour post-hypoxia. In order to determine whether the NB cells response to hypoxia also involves modulation of genes enhancing survival migration and invasion we utilise real-time PCR and analysed the expression of genes involved in these processes. In hypoxic SH-SY5Y NB cells, genes involved in maintenance of cell-cell and cell-matrix interaction (ie. APC, E-cadherin, catenin, EphB2, fibronectin-1, TIP30, TIMP4) were down-regulated by up to 90%. Under the same conditions, genes involved in enhancement of metastatic behaviour (integrin a7b1, HGF, TGFB1, VEGF, KiSS1, IL1B) were dramatically up-regulated above 200%. We have demonstrated that NB cells are able to adapt to low oxygen/hypoxia via mechanisms involving modulation of HIF-1ά expression and hypoxia responsive genes including VEGF. In addition we have for the first time also determined regulation of key genes promoting NB cell transition from an adherent phenotype to a potential highly migrating, invasive and aggressive NB cell type. Similar mechanisms might exist and contribute *in vivo* to enhancing metastatic behaviour of solid tumors, including neuroblastoma.

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CD151 GENE KNOCK-DOWN SUPPRESSES THE MOTILITY OF PROSTATE CANCER CELL LINE PC3: A LINK TO PROSTATE CANCER METASTASIS?

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Introduction: In previous work from our laboratory, we found that the tetraspanin family member CD151 has prognostic value in prostate cancer; patients with lower tumour content have a more favourable prognosis compared to patients with higher amounts of expression. Furthermore, we showed that CD151 gene over-expression promotes the motility and invasion properties of the prostate cancer cell line LNcap. To confirm the role of CD151 in prostate cancer metastasis, we set up a CD151 gene knock-down model in PC3 cells using SiRNA method.

Methods : A mixture of four different CD151 oligo SiRNAs were obtained from Dharmacon, USA. The CD151 SiRNAs were transiently transfected into the prostate cancer cell line PC3 using Oligofectamine (Invitrogen). The knock-down effect was confirmed by Western blot at 24, 48 and 72 hours time points. The cell knock-down at the 48 hour time point were chosen for migration and invasion studies using an Invasion Chamber (Invitrogen). The wild type and control SiRNA transfected PC3 cells were used as controls. We also created 2 pairs of pBabe/U6/CD151 SiRNA constructions, which can be used in permanent knock-down.

Results: The CD151 gene knock-down PC3 cells were less invasive than the wild type and control SiRNA transfected cell lines ($P<0.01$, $P<0.01$). The same trend was also found in migration ($P<0.01$, $P<0.01$). However, proliferation was not changed by CD151 gene knock-down.

Conclusion: Knocking down of CD151 gene suppresses the migration and invasion properties of the prostate cancer cell line PC3. These data suggest that CD151 plays a specific role in promoting prostate cancer cell motility and invasion.

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EVIDENCE FOR CROSS TALK BETWEEN MELANOCORTIN-1 RECEPTOR AND NR4A NUCLEAR RECEPTOR SIGNALLING IN MELANOCYTIC CELLS.

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The melanocortin-1 receptor (MC1R) is a G-protein coupled receptor that is a key regulator of melanocyte function and is known to play a significant role in the determination of diverse pigmentation phenotypes observed in mammals including humans. Peptide ligands α-MSH and ACTH binds with high affinity to MC1R promoting a functional switch to the synthesis of darker melanins primarily via a positive coupling to adenylate cyclase. This switch is most notably observed as part of the UV induced tanning response mediated by increased release of α-MSH and ACTH (among other bio-active peptides) by both keratinocytes and melanocytes. The MC1R locus has been shown to be highly polymorphic in humans with a number of functional variants being strongly associated with red hair and fair skin phenotypes. Individuals with such phenotypes have a significantly greater incidence of melanoma and non-melanoma skin cancer highlighting the importance of MC1R in the co-ordination of melanocyte photo-protective function. Delayed responses to α-MSH stimulation of melanocytes such as increased melanin content and activity of pigmentation genes are well established, however the regulatory mechanisms initiated beyond the immediate cAMP response remain poorly understood. Accordingly, we aimed to identify transcriptional control points that function within the immediate/short term following activation of MC1R. One potential candidate group is the NR4A sub-family of nuclear receptors that includes Nur77 (NR4A1), Nurr1 (NR4A2) and NOR-1 (NR4A3), genes that are known to be rapidly induced by a range of inflammatory and mitogenic stimuli in other cell types. Preliminary evidence obtained using human and mouse melanoma cell lines and primary human melanocytes has demonstrated a striking and transient immediate-early response of these genes in response to MC1R activation by NDP-MSH. Current investigations are aimed at further characterising this response and determining the functional role the NR4A genes play in melanocytic signalling.

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MALIGNANT INSULINOMAS WITH HEPATIC METASTASES SUCCESSFULLY TREATED WITH SELECTIVE INTERNAL RADIATION THERAPY (SIRT).

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Insulinomas are rare pancreatic islet cell tumours with an incidence of 4 cases per million per year. About 10% of all insulinomas are malignant. The prognosis for malignant insulinoma is poor, with 10-year survival estimated at 29%. Individuals suffer from severe debilitating and life threatening hypoglycaemia. Such episodes are worse during periods of fasting. Malignant insulinomas are poorly responsive to conventional therapy. Current therapy is therefore palliative. Surgical cure is possible if detected early enough before unresectable disease occurs. Selective Internal Radiation Therapy (SIRT) is an emerging area in radiological oncology that may improve the prognosis. SIRT in combination with intrahepatic 5-floxuridine chemotherapy has been effective in treating hepatic metastases from colorectal cancer. The therapy relies on delivering 32 micrometre spheres impregnated with yttrium-90 (90Y) injected through a femoral artery catheter, which has been positioned into the hepatic artery supplying the tumour. The beads become lodged within the tumour vessels where they exert their local radiation effects causing tumour cell death. We report 2 cases with extensive hepatic metastases that had been very difficult to manage despite aggressive treatment but had achieved a sustained clinical remission only when treated with SIRT. We believe this is the first time such a therapy has been successfully used in malignant insulinomas with hepatic metastases. Use of SIRT should be considered in other similar patients.

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CD151 – A NOVEL CLINICAL PROGNOSTIC TUMOUR MARKER IN PROSTATE CANCER.

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Introduction: Prostate cancer represents the highest incidence of cancer amongst Australian men with a lifetime risk of 1 in 11 and the second highest cause of cancer death amongst men. [1] Treatment options for intermediate Gleason grade carcinomas (Gleason 5-7) often provide a management dilemma for the treating clinician since disease progression is poorly understood and the course of the disease is often unpredictable. A new prognostic tumour marker may help change that. CD151 is a cell membrane protein from the tetraspanin family and has been shown to be over-expressed in a variety of malignancies including lung, colon, melanoma, pancreatic and prostate. [2, 3] Recent research suggests CD151 over-expression acts to increase cell motility and alters intracellular signalling pathways, both important factors in the metastatic cascade. [3-5] Furthermore, CD151 has now been shown to be a better prognostic indicator than Gleason score.[6] The present study aims to corroborate the relationship between CD151 expression in human prostate cancer with survival and to determine if a correlation exists between clinical and biochemical parameters.

Methods & Results: 190 patients with primary prostate cancer diagnosed between 1984 and 1998 were recruited retrospectively. Paraffin blocked tissue was Gleason graded then immuno-histochemically stained with anti-CD151 antibody. Quantitative measurements by MCID of staining intensity and subsequent statistical analysis will be performed on the clinical subgroups. However, preliminary immunohistochemistry suggests a correlation between CD151 over expression and poorer prognosis.

Discussion: A correlation between expression and outcome suggests the importance of CD151 as a prognostic tumour marker. In the diagnostic phase of patient work-up, CD151 may therefore prove to be a useful tool in the clinician's arsenal in determining appropriate treatment.

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MELATONIN AS AN IMMUNOENHANCING VACCINE ADJUVANT

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Melatonin has immunoenhancing effects. In particular, it enhances T helper cell activity and IL-2 production. This raises the possibility that melatonin could potentially be used as a vaccine adjuvant to enhance the immune response.

Recent attention has focused on the need for a suitable adjuvant to enhance the efficacy of both human and avian influenza vaccines. We sought to test whether oral melatonin enhanced the vaccine response to a formalin inactivated influenza vaccine (Fluvax©, CSL). To compare the adjuvant effect of melatonin, a control group was immunised with Fluvax© plus microparticulate inulin (Advax©, Vaxine Pty Ltd) which is a known effective influenza adjuvant. Mice in groups of seven were injected intramuscularly twice, two weeks apart with Fluvax© with or without Advax©. Melatonin was added in the drinking water 5 days prior to immunisation and continued 5 days post immunisation. Mice were bled 14 days after the 2nd vaccination (day 28) and serum total IgG, IgG1, IgG2a were measured by ELISA. After sacrifice spleen cells were extracted and lymphoproliferation was measured using CSFE technique. Splenocyte culture supernatants were also assayed for γ-IFN. This study provides information regarding the possibility of melatonin's role as a vaccine adjuvant to enhance the efficacy of influenza vaccine.

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IS DEFECTIVE IMMUNITY A FEATURE OF ALSTROM SYNDROME?

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Alström syndrome is a rare autosomal recessive disorder cause by mutations in *ALMS1*, a gene whose function remains unclear. This syndrome affects multiple organs and results in development of childhood obesity, metabolic disturbances (insulin resistance, type 2 diabetes, hypercholesterolaemia, hepatosteatosis), sensorineural hearing loss and infertility. The syndrome has variable clinical expression and members bearing the same mutation often present with different symptoms and at variable ages. The Fat Aussie (FATs) is an obese mouse strain with a spontaneous inactivating mutation in *ALMS1*. FATs develop similar clinical features of Alström syndrome with hyperphagic obesity, diabetes, hepatosteatosis, dyslipidemia and impair spermatogenesis. We were interested to test whether the gene, *ALMS1*, which is widely expressed in tissues throughout the body, has a role in immune function. To evaluate this, four groups of seven mice; FATs and wildtype littermates, were immunised twice (two weeks apart) with Fluvax (CSL) with or without Advax (Vaxine). Fourteen days after the 2nd injection (Day 28), the mice were bled and serum collected to measure total IgG, IgG1, 2a, 2b by ELISA. This study provides data regarding the potential immunological actions of *ALMS1*, offering further insight into its role in Alström syndrome.

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THE RELATIONSHIP BETWEEN BODY COMPOSITION AND PHYSICAL PERFORMANCE: A STUDY IN YOUNG RECREATIONAL ATHLETES.

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To understand the relationship between body composition and physical performance we studied 84 recreational athletes (60 m, 24 f) aged 18-40, exercising ≥ 2 times/week for ≥ one year. Performance was assessed by 4 parameters: a) sub-maximal cycle test for VO_2max, b) dead lift dynamometry for maximal strength, c) single vertical jump for maximal power, and d) cycle Wingate test for anaerobic sprint capacity. Lean body mass (LBM) and fat mass (FM) were measured by DEXA and data analysed by simple and multiple linear regression. In the entire cohort, LBM correlated positively with all performance measures (r^2=0.50-0.83, p<0.001). FM correlated negatively with jump height only (r^2=0.10, p=0.002). All performance measures and LBM were higher in men. In men, LBM positively correlated with all measures (r^2=0.15-0.55, p<0.001) except jump height, while FM correlated negatively with jump height only (r^2=0.16, p<0.001). In women, LBM positively correlated to all performance measures (r^2=0.23-0.73, p<0.001) whereas FM was not related to any.

	VO_2max [L/min]	Dead lift [kg]	Jump Height [cm]	Total Work [kJ]	LBM [kg]	FM [kg]
Male	3.8±0.8	191.4±35.2	55.1±8.1	22.8±3.8	63.9±8.3	16.4±8.1
Female	2.3±0.6*	123.1±24.6*	35.4±7.5*	12.7±3.2*	40.3±5.2*	18.9±5.4
Mean±SD; *: p<0.001 vs male						

In a multiple regression LBM accounted for >50 % (r^2=0.50-0.83, p<0.001) of the total variance for all measures while FM negatively predicted dead lift (r^2=0.05, p<0.02) and jump height (r^2=0.15, p<0.001) only. Gender exerted a minor but significant effect on jump height only (r^2=0.03, p<0.02)

In summary, LBM had a positive correlation and stronger relationship with all performance measures than FM, which negatively correlated with dead lift and jump height. The influence of gender was minor after accounting for differences in body composition. In conclusion, in recreational athletes, body composition and physical performance are closely interrelated and gender differences in most performance parameters could be attributed to differences in body composition. Prospective studies are required to address whether growth hormone-induced changes in body composition alter performance. (Supported by the World Anti-Doping Agency and Australian Government Anti-Doping Research Program.)

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LEGUMAIN IN EARLY BOVINE PLACENTATION

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We examined the maternal-embryo interactions during the early endometrial apposition of the trophoblast by comparing the proteins present at Day 17 in uterine luminal fluid (ULF) from pregnant and non-pregnant cows using 2D-gel electrophoresis. Three protein spots that were presentat reduced levels in pregnant ULF were identified by Mass spectrophotomtry (MALDI-TOF) as post translational isoforms of legumain.1 Legumain, a lysosomal cysteine endopeptidase that specifically cleaves on the carboxyl side of asparagine residues is particularly abundant in mouse kidney and placenta. 2 Legumain throughout the estrous cycle and in early pregnancy was examined using a beef tissue library of endometrial samples collected throughout the estrous cycle and Days 1-31 of pregnancy (n=2 per day).

Legumain mRNA was present in low amountsin endometrial tissue early in the estrous cycle, increasing three-fold on Days 13 and 14 then dropping, from Day 16, to low levels by Day 20. The pregnant endometrium followed the same expression pattern until Day 18 when expression of legumain was 1.5-2 times greater than that of non-pregnant uteri, returning to low levels between Days 26-31 of pregnancy.

Western blotting of proteins from Day 18 endometrial tissue revealed the presence of the 56kDa proform of legumain as well as three higher molecular weight bands. Day 18 ULF proteins contained the 56kDa proform and the 46kDa active form of legumain. More legumain was present in non-pregnant than in pregnant ULF from empty horns than in the gravid horns.

A function of legumain is to activate other protease zymogens by proteolytic cleavage of an asparaginal bond, therefore, its up-regulation in expression during the peri-implantation period may suggest an involvement in the modulation of protease activity necessary for successful invasion of the endometrium.

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